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HYPRESSIN®

perindopril

NOVO

IZNAD OČEKIVANJA

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(br. rješenja: 04-07.3-1-3222/15)
Tablete, 30 x 4 mg
(br. rješenja: 04-07.3-1-3223/15)
Tablete, 30 x 8 mg
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Bosnalijek d.d., Jukićeva 53, Sarajevo, BiH

ODOBRENE INDIKACIJE: Hipertenzija; zatajenje srca; stabilna bolest koronarnih arterija.

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Đorđe Mihajlović (1875-1919), "Old Tuzla", 1918, watercolor, 320x250 mm. Courtesy of the Museum of Eastern Bosnia, Tuzla, BA.

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The Reliability and Validity of the Three Modified Versions of the Children's Fear Survey Schedule-Dental Subscale of 9-12 Year Old Children in a Clinical Setting in Bosnia and Herzegovina

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Key words: Dental fear and anxiety ▪ Children ▪ CFSS-DS scale ▪ Modified versions ▪ Reliability and validity.

Objectives. This study sought to obtain a comprehensive, reliable and valid instrument for evaluation of the presence of dental fear and anxiety (DFA) in children, through evaluation of the reliability and validity of three modified versions of the Children's Fear Survey Schedule-Dental Subscale (CFSS-DS). **Materials and Methods**. The study sample comprised children aged 9, 10, 11 and 12 years. The first sample group (200 patients) filled in a modified version of the CFSS-DS scale, the second sample group (100 patients) filled in a short form of the Dental Fear Survey Schedule, and the third sample group (100 patients) filled in a short version of the CFSS-DS scale, prior to dental treatment, respectively. In order to determine test-retest reliability, the 184 patients from the first sample group filled in the modified version of the CFSS-DS scale again, prior to their next scheduled dental appointment. **Results**. The modified version of the CFSS-DS scale had the best internal consistency reliability (α=0.907), as well as validity results, compared to the other two instruments used. Test-retest reliability was moderate (Intraclass correlation coefficient: 0.58). **Conclusions**. Of the three psychometric instruments used for evaluation of DFA presence in children, the modified version of the CFSS-DS scale showed the most clinically adequate reliability and validity values. This study thus provides a new psychometric instrument that should be considered for clinical use in evaluation of DFA presence in 9-12 year-olds, in a clinical setting sample type of children.

Introduction

To date, the instrument most used for measuring dental fear and anxiety (DFA) in children has been the Children's Fear Survey Schedule-Dental Subscale (CFSS-DS), also known as the Dental Fear Survey Schedule for Children (DFSS-C). The CFSS-DS scale was designed by Cuthbert and Malamed in 1982, and was based on a wider instrument for measuring fear in children, the Children's Fear Survey Schedule (CFSS) (1), designed in 1967 by Scherer and Nakamura, and also known as the Fear Survey Schedule for Children (FSS-FC) (2).

The CFSS-DS scale has 15 questions (items) covering dental (invasive and non-

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invasive treatment procedures), medical and general situations. The answers range on a Likert scale from 1 (not afraid) to 5 (very afraid), with a total scale score between 15 and 75. There are parent and child versions of the CFSS-DS scale, depending on who answers the questions, the child or a parent (1). These two versions of the scale have been used in numerous studies, in patients aged from 3.5 to 19 years, for evaluation of DFA presence, in many countries (3-9). This scale was the most preferred in many studies compared with other measuring instruments for evaluation of DFA presence, due to its better psychometric characteristics and wider range of DFA related aspects. The CFSS-DS scale has good reliability but variable validity (4-9). In a previous study it was determined that the CFSS-DS scale had good internal consistency reliability (Cronbach α=0.861). Factor analysis revealed four factors that together explained the 63.79% variance of results (6).

However, some CFSS-DS scale validity results in several studies conducted showed that some of the scale items did not contribute much to explaining the variance of results (4-9). As a result, some shortened modifications of the CFSS-DS scale have appeared. Carson and Freeman suggested a shorter form of the CFSS-DS scale with 8 scale items in 1997, and in 2005 Rantavuori et al. also published studies with a shortened CFSS-DS scale of 10 scale items, and in 2004 and 2012 there were studies with a shortened scale of 11 scale items. The solid variable normative value of these scales has been reported, with various limitations and suggestions for further research (4, 10-12). This showed that the authors tended to correct the disadvantages of the CFSS-DS scale and to create an instrument that covers all aspects of DFA presence, but mostly by shortening it (4, 10-12). Also, the previous research showed that there is room for managing the content of the scale to improve its capability of differing between children with and those without DFA (6).

Therefore, the aim of this study was to obtain a single comprehensive, reliable and valid instrument for evaluation of DFA, in order to measure more precisely its presence in children, to determine its reliability and validity values, and to compare it with the other two shortened versions of the CFSS-DS scale.

Patients and methods

Participants and study design

The study sample was clinical and comprised of a specific population of children, who were randomly selected patients who attended the Clinic of Preventive and Pediatric Dentistry of the Faculty of Dentistry of Sarajevo University, in the period between January and October 2013. The reason for their visit to the Clinic was a permanent tooth carious lesion of medium depth (dentinal depth of the lesion corresponded with ICDAS code 5), which had to be resolved according to the dental treatment plan. This reason was the main criterion for inclusion in the study, in order to avoid other potential biases in the clinical setting. The patients were aged 9, 10, 11 and 12 years. The reason for choosing this age group was the general cognitive and psychosocial development of the children (13, 14). In order to avoid selection bias, child behavior in the dental office was evaluated and medical history was determined where it was necessary, so that children with general psychological problems could be excluded from the study sample. The patients' parents signed an informed consent form for the participation of their children in this study, and the assent of the child patients was also obtained.

Prior to the scheduled dental treatment, clear instructions were given to the child participants regarding participation in the

study, and the patients provided answers to the three modifications of the CSSS-DS scale in the dental office, as follows: a modified version of the Children's Fear Survey Schedule-Dental Subscale, the Carson and Freeman Short Form of the CFSS-DS scale (4) and the 10-item Short Version of CFSS-DS scale designed by Rantavuori et al. (10), respectively. This was undertaken in order to compare the reliability and validity values of these three modifications of the CFSS-DS scale. Accordingly, the sample consisted of three groups, where the patients were assigned randomly to one of them. The number of patients in the groups was determined according to the statistical minimum that was sufficient for conducting analyses of the reliability and validity values of these scales (15). So, the child participants responded to the scale questions by themselves, without any help from the researchers or their parents. When the children finished, the researchers immediately collected the questionnaires from the child participants, just before the scheduled dental treatment started.

The first sample group of 200 patients filled in the modified version of the Children's Fear Survey Schedule-Dental Subscale (CFSS-DS-mod scale). The CFSS-DS-mod scale was revised in its design in comparison with the original CFSS-DS scale as follows: it was reduced by removing items that were not related to the dentist or the treatment, and at the same time extended with new dental (treatment) situations which were not in it originally. The reason for this modification was the previous research with the original CFSS-DS scale, where it was determined that some of the scale items did not improve its normative value, and also the fact that not all clinical dental situations were included in this instrument (6). The previous studies by others had also led to this conclusion (4-5, 7-9). As a result, a new scale was created, the CFSS-DS-mod scale, originally in the Bosnian language. For the purpose of this publication, the scale items were translated into English by a licensed translator (Table 1), and back-translated into Bosnian in order to avoid any bias in the meaning of the items.

The CFSS-DS mod scale had 17 items (questions) which were adapted to the patients according to their meaning. The idea was to include all situations related to the dentist, the dental office and dental treatment. So, the CFSS-DS-mod scale design comprised four categories, as follows: general terms (dentist), being in the waiting room and in the dental office itself, various forms and stages of dental treatment, as well as the notion of dental pain. Eight items from the original CFSS-DS scale remained, but two of these were combined in the new scale. The meaning of two of the original items was changed, so that eight new items were added to the scale. These items were also first translated by a licensed translator for English language, and back-translated into Bosnian prior to their use. The answers to this new scale questions ranged on a 5-point Likert scale from 1 (not afraid) to 5 (very afraid). The total CFSS-DS-mod scale score ranged from 17 to 85.

The second sample group of 100 patients filled in the Short Form of the Dental Fear Survey Schedule (DFSS-SF) (4). It consisted of eight items. For the purpose of this study the DFSS-SF scale items were first translated into English and back-translated into Bosnian by a licensed translator, prior to their use, in order to avoid any potential bias in the meaning of the items. Similarly to the previous scale, eight items of the DFSS-SF scale were excluded from the original CFSS-DS scale, and one item about tooth extraction was added (4). The answers to the questions were coded by the 5-point Likert scale, from 1 (not afraid) to 5 (very afraid). The total score ranged from 8 to 40 (4).

The third sample group of 100 patients filled in the Short Version of the CFSS-DS

Table 1. The Modified Version of the Children's Fear Survey Schedule-Dental Subscale

Questions		Not afraid	Slightly afraid	Fairly afraid	Quite afraid	Very afraid
1.	Are you afraid of being in the waiting room before entering the dental office?	1	2	3	4	5
2.	Are you afraid of being in the dental office?	1	2	3	4	5
3.	Are you afraid of the dentist?	1	2	3	4	5
4.	Are you afraid of people in white uniforms?	1	2	3	4	5
5.	Are you afraid of sitting in the dental chair?	1	2	3	4	5
6.	Are you afraid when the dentist examines your mouth with dental instruments?	1	2	3	4	5
7.	Are you afraid when you keep your mouth open?	1	2	3	4	5
8.	Are you afraid of the suction device in your mouth?	1	2	3	4	5
9.	Are you afraid when the dentist cleans your teeth from dental plaque?	1	2	3	4	5
10.	Are you afraid when your teeth are being drilled?	1	2	3	4	5
11.	Are you afraid of the sound of dental drilling devices?	1	2	3	4	5
12.	Are you afraid of the sight of dental drilling devices?	1	2	3	4	5
13.	Are you afraid of the dental syringe?	1	2	3	4	5
14.	Are you afraid of the dental syringe needle?	1	2	3	4	5
15.	Are you afraid of tooth extraction?	1	2	3	4	5
16.	Are you afraid of dental treatment that causes pain?	1	2	3	4	5
17.	Are you afraid when you are unable to breathe during dental treatment?	1	2	3	4	5

scale (CFSS-SV) (10). It consisted of 10 items. For the same reasons as with the other two scales used in this study, translation into English language and back translation into Bosnian was undertaken by a licensed translator. In the design of the CFSS-SV scale seven items were excluded from the original CFSS-DS scale, and two items were added about pain during dental treatment and the presence of a suction device in the mouth (10). The answers to the questions were coded by the 5-point Likert scale, from 1 (not afraid) to 5 (very afraid). The total score ranged from 10 to 50 (10).

In order to determine the test-retest reliability of the CFSS-DS-mod scale, a retest evaluation of DFA presence by this scale had to be conducted. According to the previously established dental treatment plan, 184 patients from the first group of 200 patients, who came back to resolve a carious lesion of a permanent tooth of medium depth, answered the same CFSS-DS-mod scale questions again, under the same dental office conditions, prior to their scheduled treatment. This was undertaken after one month from their first study test sample visit, consecutively for each patient. The rest of the 16 patients missed their scheduled appointment and were excluded from the study retest sample.

Ethics statement

This research was approved by the Ethical Committee of the Faculty of Dentistry of Sarajevo University, and also was conducted according to the Declaration of Helsinki (16).

Statistical analysis

The results obtained from this study were statistically processed as follows:

- Descriptive values were presented by descriptive statistics (frequency of the study participants by their age in percentages, mean values and standard deviations of age, and of the scores of the different scales used in the study sample groups);
- The Cronbach α coefficient was calculated as the internal consistency reliability of the three scales, altogether with the corrected item-total correlations presented for each of scale items;
- The test-retest reliability of the CFSS-DS-mod scale was determined by calculating an intra-class correlation coefficient (ICC);
- The construct validity of the three scales was determined by explorative factor analysis (EFA) applying varimax rotation. The criteria for proper factor analysis were determined by the Kaiser-Meyer-Olkin test of sampling adequacy (KMO) and the Bartlett test of sphericity (Bartlett). Percentage and cumulative percentage of explained variance, as well as determined Eigen values, were presented.

All statistical analyses were obtained for a $P<0.05$ significance level in the IBM Statistical Package for Social Science software (version 23.0) for the Windows operative system.

Results

The descriptives of the study sample were as follows: The first sample group contained 58 (29%), 44 (22%), 53 (26.5%) and 45 (22.5%) patients aged 9, 10, 11 and 12 years, respectively. The mean group age was 10.43±1.13 years. The mean CFSS-DS-mod scale score was 27.52±9.21. The second sample group

Table 2. Internal consistency reliability of CFSS-DS-mod, DFSS-SF and CFSS-SV scales

CFSS-DS-mod scale		DFSS-SF scale		CFSS-SV scale	
(n=200)		(n=100)		(n=100)	
Cronbach α=0.907	Corrected item-total correlations	*Cronbach* α=0.650	Corrected item-total correlations	*Cronbach* α=0.800	Corrected item-total correlations
Item 1	0.536	Item 1	0.592	Item 1	0.553
Item 2	0.672	Item 2	0.311	Item 2	0.420
Item 3	0.421	Item 3	0.497	Item 3	0.335
Item 4	0.243	Item 4	0.342	Item 4	0.618
Item 5	0.655	Item 5	0.113	Item 5	0.573
Item 6	0.657	Item 6	0.267	Item 6	0.427
Item 7	0.465	Item 7	0.475	Item 7	0.177
Item 8	0.445	Item 8	0.474	Item 8	0.658
Item 9	0.595	-	-	Item 9	0.410
Item 10	0.670	-	-	Item 10	0.671
Item 11	0.687	-	-	-	-
Item 12	0.652	-	-	-	-
Item 13	0.510	-	-	-	-
Item 14	0.583	-	-	-	-
Item 15	0.582	-	-	-	-
Item 16	0.763	-	-	-	-
Item 17	0.618	-	-	-	-

Table 3. Validity results of the three modifications of the CFSS-DS scale

CFSS-DS-mod scale			DFSS-SF scale			CFSS-SV scale		
(n=200)			(n=100)			(n=100)		
KMO =0.880			KMO=0.562			KMO=0.763		
Bartlett P<0.0005			Bartlett P<0.0005			Bartlett P<0.0005		
Items	Factors:*		Items	Factors:*		Items	Factors:*	
	1	2		1	2		1	2
Item 10	0.795	0.212	Item 7	0.835	0.099	Item 10	0.776	-0.127
Item 9	0.716	0.195	Item 8	0.791	0.109	Item 8	0.772	0.172
Item 2	0.710	0.309	Item 1	0.626	0.237	Item 4	0.744	-0.094
Item 6	0.658	0.339	Item 4	0.618	-0.017	Item 1	0.693	0.291
Item 5	0.621	0.382	Item 2	0.449	-0.046	Item 5	0.684	-0.029
Item 16	0.617	0.520	Item 5	-0.193	0.750	Item 6	0.572	0.091
Item 11	0.595	0.459	Item 3	0.455	0.715	Item 2	0.518	-0.098
Item 12	0.549	0.454	Item 6	0.101	0.673	Item 9	0.510	-0.276
Item 3	0.511	0.140	-	-	-	Item 7	0.254	0.694
Item 1	0.503	0.344	-	-	-	Item 3	0.422	-0.615
Item 4	0.455	-0.074	-	-	-	-	-	-
Item 14	0.099	0.828	-	-	-	-	-	-
Item 13	0.014	0.817	-	-	-	-	-	-
Item 15	0.252	0.667	-	-	-	-	-	-
Item 17	0.422	0.539	-	-	-	-	-	-
Item 7	0.277	0.483	-	-	-	-	-	-
Item 8	0.242	0.482	-	-	-	-	-	-
% Variance	27.122	22.490	-	31.910	20.084	-	37.973	10.934
% Cumulative	27.122	49.613	-	31.910	51.995	-	37.973	48.907
Eigen value	7.027	1.407	-	2.794	1.366	-	3.799	1.091

KMO=Kaiser-Meyer-Olkin test of sampling adequacy; *The loading factors of scales Items are presented by their descending value.

contained 26 (26%), 15 (15%), 24 (24%) and 35 (35%) patients aged 9, 10, 11 and 12 years, respectively. The mean group age was 10.68±1.21 years. The mean DFSS-SF scale score was 10.71±2.83. The third sample group contained 25 (25%), 27 (27%), 25 (25%) and 23 (23%) patients aged 9, 10, 11 and 12 years, respectively. The mean group age was 10.46±1.1 years. The mean CFSS-SV scale score was 15.82±5.17.

Table 2 shows the results of internal consistency reliability, with corrected item-total correlations for the three modifications of the CFSS-DS scale. It was shown that the Cronbach coefficient had the highest value in the CFSS-DS-mod scale (α=0.907). The corrected item-total correlations also had the highest values in this scale. The lowest internal consistency reliability was found using the DFSS-SF scale (α=0.650), and in the CFSS-DS-mod scale the lowest corrected item total correlation was for the 4th item (fear of people in white uniforms; r=0.243). The first retest sample group showed drop-out of 8% (16 of the 200 patients). Test-retest reliability showed a value of ICC=0.58,

P<0.0005. No statistically significant differences were determined between the study participants and those who dropped out.

Table 3 shows the results of explorative factor analysis (EFA) with Varimax rotation for the three modifications of the CFSS-DS scale, with KMO and Bartlett test values. All three scales obtained two-factor solutions, with the Eigen values of extracted factors which were greater than 1. The total variance explained was around 50% (from 48.907% to 51.995%). Although the DFSS-SF and CFSS-SV scales showed two-factor solutions with a solid percentage of the variance of its results explained, in the case of the DFSS-SF scale, KMO was 0.576, and also in the CFSS-SV scale, in the second factor, the 3rd item had negative factor loading (r=-0.615). On the other hand, in the CFSS-DS-mod scale, two factors were also extracted, which explained 49.613% of the variance of results obtained with this scale. The first factor was related mostly to the dentist and dental staff, the dental office and its contents, as well as non-invasive dental procedures. The second factor was mostly related to invasive dental procedures.

Discussion

The internal consistency reliability estimated with the CFSS-DS-mod scale demonstrate its excellent reliability value according to the α ranking evaluation (≥0.9). However, the relatively low corrected item-total correlation for question no. 4. "Fear of people in white uniforms", would suggest that this item was perhaps in disparity with the list of factors for DFA appearance in the CFSS-DS-mod scale. All the other items had a value above 0.3. On the basis of this and the results of factor analysis, we suggest that the CFSS-DS-mod scale measures mostly the same underlying concept, the presence of DFA in children. Test-retest reliability in contrast showed a moderate ICC value. The test reliability and reproducibility in general usually depends upon many factors, such as sample size and quality, instrument design and its homogeneity, the retest period, etc. Although this reliability should be considered as questionable, since the usual acceptable threshold value is 0.7, sometimes authors regard a test-retest reliability threshold value of 0.6 as possible (15, 17).

Although the DFSS-SF and CFSS-SV scales served more as controls, they did show lower internal consistency reliability values. They also had a few corrected item-total correlations values below 0.3, and this decreased with the increase of the number of items per scale.

We have already emphasized that the situations related to the dentist and the staff, the dental office and its contents, as well as non-invasive dental procedures mostly had two-factor solutions of the CFSS-DS-mod scale as the first factor. So, it seemed that the most provoking factors for the presence of DFA tended to create one side of its two-dimensional concept, putting every other component on the other side. However, the high first factor loadings also had items such as fear of tooth drilling, as well as fear of pain caused by dental treatment. Preparation of a carious lesion is certainly an invasive dental procedure, but it affects body integrity less than two other well-known dental interventions – tooth extraction and dental local anesthesia administration. So, it should not be surprising that these three were separated from one another. The fear of pain caused by dental procedures also had high second factor loadings, so this item by nature should probably belong to the second factor.

The results of EFA for the DFSS-SF and CFSS-SV scales were questionable. In order for the conditions for proper conduct of the EFA to be met, KMO had to be ≥0.6, and the Bartlett test of sphericity (Bartlett) had to be P<0.05 (15). In the case of the DFSS-SF scale this condition of the KMO minimal value

Table 4. Review of the studies where the CFSS-DS scale and its modifications were used and its reliability and validity values were determined

Authors:	Year	Country	Scale		Examinees			Reliability	Construct validity	
			Name:	Informant	Number	Age (years)	Sample type:	Type	Number of factors	% of explained variance
Alvesalo et al. (18)	1993	Finland	CFSS-DS	Child	828	11-14	School	α=0.85	3	54
Klingberg (19)	1994	Sweden	CFSS-DS	Parent	52	4-14	Clinical (with DBMP)	ρ=0.97(***)	–	–
Milgrom et al. (20)	1994	Canada	CFSS-DS	Parent	70	5-15	Clinical	α=0.87	4	67.6
		China			99	2.5-7		α=0.90	3	64.5
Ten Berge et al. (21)	1998	Holland	CFSS-DS	Parent	150	4-12	Clinical	α=0.90	3	65
Majstorović et al. (22)	2003	Croatia	CFSS-DS	Parent	165	5-15	Clinical (with DFA)	α=0.83	–	–
Nakai et al. (23)	2005	Japan	CFSS-DS	Child	134	8-15	Clinical	α=0.91	–	–
					532		Clinical	α=0.86	3	54.8
					1250		School	α=0.89	–	–
Arapostathis et al. (24)	2008	Greece	CFSS-DS	Parent	260	4-12	Clinical	α=0.85; ρ=0.71(***)	–	–
Lee et al. (25)	2009	Taiwan	CFSS-DS	Parent	1819	5-8	Preschool, school	α=0.90	3	–
Bajrić et al. (6)	2010	B&H	CFSS-DS	Child	120	8, 12, 15	Clinical	α=0.86	4	63.79
Singh et al. (8)	2010	India	CFSS-DS	Child	197	7-12	Clinical	α=0.92	3	64.7
Ma et al. (7)	2014	China	CFSS-DS	Child	206	6-10	Clinical	α=0.85; ρ=0.71(***)	3	53.7
El-Housseiny et al. (9)	2016	Saudi Arabia	CFSS-DS	Child	1546	6-12	School	α=0.88 ICC=0.83(***)	3	53.47
Carson and Freeman (4)	1997	Northern Ireland	DFSS-SF	Child	100	5-11	Clinical	K=0.69-0.96	2	62-64
Folayan and Otuyemi (26)	2002	Nigeria	DFSS-SF	Child	30	8-13	School Clinical	α=0.82; ρ=0.73(***)	–	–
Rantavuori et al. (10)	2005	Finland	CFSS-SV	Parent	1212	6, 9, 12, 15	Clinical	–	2	60.59-63.85

DBMP=Dental Behavior Management Problems; ***P<0.0005.

was not achieved, so there were no minimal statistical reasons for this factor analysis to be considered appropriate. On the other hand, the negative second factor loading of one of two items made the CFSS-SV scale two-factor solution unsustainable for at least two reasons. The first reason is that the loading was negative, which meant that this item explained the second factor in the opposite way. If we were to exclude it from the second factor, then only one 7th item would remain. The extracted factor with only one item in it would not be statistically possible (15). These are the reasons why we should not consider the construct validities of the DFSS-SF and CFSS-SV scales as proper ones.

In Table 4 several studies are shown of evaluation of DFA presence, where the reliability and validity values of the CFSS-DS scale used and its modifications are presented. Besides different methodologies (sample size, age and type, type of informant), compared to the original CFSS-DS scale, the modified scale version from this study had mostly higher α values of internal consistency reliability (6-9, 18-25). This

was also the case compared to the DFSS-SF and CFSS-SV scales (which we confirmed), although fewer studies have been conducted (4, 10, 26). In contrast, the original scale and its two modifications showed better construct validity concerning the % of total variance explained, but the number of extracted factors remained variable (4, 6-10, 18, 20-21, 23). Nevertheless, the over 50% of variance in our scale remained unexplained. Although the original design of the CFSS-DS-mod scale had four categories, the scale items were latently grouped into two factors. The number of 200 cases for conducting the EFA was statistically minimal, and sample size is one of the most important conditions for its proper execution (15).

Limitations and improvements

The results obtained from this study showed some limitations (possible selection bias of patients in the clinical setting, and no comparison with the original design of the CFSS-DS scale, for example), as well as ways of improving and further analysis of the CFSS-DS-mod scale, in terms of a larger and wider sample size with a non-clinical setting sample type, type of informant, conducting confirmatory factor analysis, cut-off score evaluation, socio-economic state, etc., so that the normative values of this scale could be evaluated in the general population.

Conclusion

This study provided a new psychometric instrument for evaluation of DFA presence in 9-12 year olds, in a clinically setting sample type of children, in the form of the CFSS-DS-mod scale. This instrument showed good reliability and validity values, and therefore should be considered for clinical use in determining DFA presence in children.

What is already known on this topic

Dental fear and anxiety is a widespread clinical phenomenon which may seriously interfere with dental treatment. The instrument used by far the most for measuring dental fear and anxiety in children is the Children's Fear Survey Schedule-Dental Subscale.

What this study adds

The results from our study provide a new instrument based on the Children's Fear Survey Schedule-Dental Subscale, for evaluation and validation of the presence of dental fear and anxiety in children.

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The Incidence and Frequency of Various Causes of Angioedema in Emergency Medicine

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Key words: Angioedema ▪ Emergency ▪ Bradykinin ▪ Icatibant ▪ Plasma-derived C1-inhibitor.

Objective. Angioedema (AE) is a potentially life-threatening event. We investigated the etiology of AE, with the emphasis on bradykinin-induced angioedema treatment in emergency medicine. **Methods.** The retrospective study included 237 patients with AE, who were examined and treated in two hospitals (group A and B) in Croatia from 2009 to 2016. The location and duration of AE, data about chronic diseases and treatment, potential causative agents (food, drugs, insect bites and chemicals), physical examination data and the subsequent treatment were analyzed. **Results.** There was no statistical difference regarding age or comorbidities but there was a statistically significant difference in etiology between the groups (Chi-square, P=0.03). Renin-angiotensin-aldosterone system (RAAS) blocker induced AE was the main cause of emergency attendance in group A (37.5%) and among the leading causes in group B (18.8%). Bradykinin-induced AE (hereditary angioedema (HAE) and RAAS-AE) were the leading causes in a total of 75 (31.5%) patients. RAAS-AE was treated with glucocorticoids and antihistamines. HAE attacks in both groups (2/7 patients, 1.5/6%) were treated with specific therapy. Other causes of AE in groups A/B were insect bites (15/23 patients, 13.5/20%), use of antibiotics/analgetics (11/17 patients, 9/15%), gastroesophageal reflux disease (10/11 patients, 8/9%), neoplasms (5/6 patients, 4/5%) and idiopatic (32/31 patients, 26.5/26%). 21% of patients were hospitalized. **Conclusion.** Bradykinin-mediated AE was the main cause of emergency attendance associated with AE. Advances in the treatment of HAE, with case reports of patients with RAAS-AE treated with C1 esterase inhibitor concentrate or bradykinin receptor antagonist, may prove to be a new, reliable and efficacious therapy option.

Introduction

Quinckes edema or angioedema (AE) is an transient, localized swelling of the deep dermis or subcutaneous/submucosal tissues (1). Angioedema usually manifests in the upper airway, and the head and neck region (1, 2). Histamine and bradykinin are two main mediators involved in AE occurrence, inducing endothelial cell permeability (3-7). Histamine-mediated AE with recognizable triggers, such as an insect bite, food, medication, and rapid onset of swelling, are often accompanied by urticaria and itching (3). Bradykinin-induced angioedema, including hereditary (hereditary angioedema (HAE) types I (due to low level of C1 ester-

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ase inhibitor (C1 INH)) and II (dysfunctional C1 INH), or HAE with normal C1 INH) or nonhereditary forms (nonallergic angiotensin-converting enzyme inhibitors (ACEI) or angiotensin II receptor blockers (ARBs) induced angioedema and acquired AE due to C1 INH deficiency) with a history of recurrent swelling and abdominal pain, are not associated with urticaria (3, 8, 9). Differentiation is essential for treatment planning since bradykinin mediated angioedema does not respond to conventional antihistamine and corticosteroid therapies (8). Angioedema due to exposure to external agents (insect bites, food, environmental allergens) is very common. Including gastrooesophageal reflux disease (GERD), it is usually identified by the patients themselves (10). The role of GERD in the development of angioedema is still controversial, especially in children (10, 11). Some tumors may produce biogenic amines, such as histamine, and release them into the circulation causing angioedema (12). Acquired AE with low C1-INH is connected with autoimmunity or malignant lymphoproliferative disorders (13).

Drugs may induce AE by three mechanisms: an allergic IgE-mediated reaction accompanied by urticaria (such as beta-lactam antibiotics); non-allergic, such as AE induced by aspirin and nonsteroidal anti-inflammatory drugs (NSAID); and the most frequent (10-25% of all cases, never associated with urticaria) including inhibition of bradykinin degradation, induced by renin-angiotensin-aldosterone system (RAAS) blockers, such as ACEI or ARBs (10, 14, 15). The RAAS plays an important role in the regulation of kidney blood flow and blood pressure, with the emphasis on angiotensinogen, which is changed from angiotensin I to angiotensin II by the angiotensin I-converting enzyme (ACE) (known as kininase II). Angiotensin II is a key factor for the inactivation of bradykinin which originates from the degradation of kininogen by kallikreins. The metabolism of bradykinin does not depend only on ACE. Several other enzymes are involved and a deficiency of any of these enzymes (due to a genetic polymorphism) will increase the risk of developing AE (15-18). This has been observed in black and Hispanic races (6, 15, 19, 20). AE with ACEI therapy (ACEI-AE) is more frequent than for other RAAS blockers, and is between 0.1% and 2.2% (6). The development of edema in the upper respiratory tract is unpredictable. The management of ACEI-AE begins by ceasing administration of ACEI as the suspected drug (6, 21). It is important to tell the patient not to take any RAAS drugs ever again, such as ARBs, while 4% of patients who have had ACEI-AE will develop AE when they switch to ARBs (22). The literature suggests that some other drugs that inhibit the mammalian target of rapamycin (mTOR inhibitors), if taken with ACEI, affect the incidence of ACEI-AE (23).There is no approved treatment for this potentially life-threatening situation (22, 24). As Craig et al. reported, specific drugs, such as a C1-INH concentrate and bradykinin B2 receptor blocker icatibant, which have been approved for acute attacks of HAE type 1 or 2, successfully resolved attacks of different types of bradykinin-induced AE (8, 25, 26).

The goal of this study was to determine the incidence of AE, and whether there is a difference in the causes and frequency of AE as a major reason for emergency attendance, with special emphasis on bradykinin-induced angioedema frequency and treatment.

Materials and methods

A retrospective, two-center analysis was conducted of all patients with AE found in the Emergency Units' medical databases. The study included a total of 237 patients with AE who were examined in the Emer-

gency Units of two hospitals in Croatia: Merkur Clinical Hospital, Zagreb (120 patients in group A) and the Department of Otorhinolaryngology, Šibenik General Hospital, Šibenik (117 patients in group B) from January 2009 to January 2016. Anamnestic findings (location and duration of AE), data about chronic diseases, treatment (primarily exposure to ACEI, ARBs) and potentially causative agents (food, drugs, insect bites and chemicals) along with the physical examination data and subsequent treatment, were analyzed for each patient with AE. As Zingale at al. proposed, ACEI-AE was diagnosed when angioedema was repeatedly present during ACEI therapy and ended upon withdrawal of the medication (10).

Since there is still no standardized severity scoring system for AE, we graduated AE as grade 0= no local reaction; grade 1= mild (isolated local reaction of the skin or mucosa); grade 2= intermediate (involves more distant skin; upper airway); grade 3= severe (potentially life-threatening condition manifesting with laryngeal symptoms); grade 4= severe multi-system reaction. A composite score was calculated using the visual analog scale, with intensity ranging from 0 to 10 (higher scores indicating more severe symptoms), using the average of the measurements for the six symptoms (pain, shortness of breath, dysphagia, change in voice, sensation of a foreign body, feeling of pressure) as proposed by Bas (7).

Statistical analysis

Data were described using descriptive statistical methods. Differences in categorical variables were tested with the Chi-square test. The normality of the distribution of numerical variables was tested by Shapiro-Wilk's test. All P values were two-sided. The level of significance was set at Alpha =0.05. The statistical analysis was performed using STATISTICA 13.1.

Results

The median age in groups A and B was 51.7 years, with 50.5% males (Table 1). The graduation of AE in this study was generally mild, characterized by mean severity scores of 1 or 2 (20%) on a scale of 0 to 4. The mean composite score on a visual-analogue scale of 0 to 10 was approximately 3.

The etiology of AE is shown in Table 1. Bradykinin-induced AE (ACEI/ARB and HAE) was the leading known cause of AE in the investigated group, in a total of 75 (31.5%) patients. AE caused by ACEI/ARB blockers was present in 67 (28%) patients. Patients with ACEI-AE were older and there were more females represented (70.5% female; mean age, 63.4±11.5 years) when compared to all AE patients. The locations of AE included swelling of the face and lips (48.1% of patients), tongue (37.5%), larynx (12.5%) and upper airways (2.9%). The duration of exposure to ACEI was between 1 and 721 days with a median of 452 days. Duration of AE symptoms was from 2 to 5 days. All patients with ACEI–AE had normal C1-inhibitor levels. There was no statistically significant difference regarding age or comorbidities (hypertension, diabetes, heart failure, kidney disease) but there was a statistically significant difference in etiology between the groups (Chi-square, P=0.03). All the patients were under the appropriate therapy for comorbidities. In group A the patients used drugs in their chronic therapy more than in group B (such as diuretics in fixed combination with ACEI, such as ramipril in 80%). All investigated patients with symptoms of AE, except HAE patients (3.8%), were treated with antihistamines, corticosteroids and epinephrine if needed (Table 1.).

Twenty-one patients (8.9%) diagnosed with GERB were also treated with proton pump inhibitors (PPI) intravenously. Fresh frozen plasma was administered success-

Table 1. Demographic and clinical characteristics of patients with angioedema

Characteristics	Group A	Group B	Treatment	Total	P*
Gender (N; %)					
Male	62 (51.7)	60 (51.3)	-	122 (51.5)	0.95
Female	58 (48.3)	57 (48.7)	-		
Age (Year, Median, IQR)	52.1 (41.8 – 63)	50.8 (40.2 – 65)	-	51.7 (40 – 60)	0.34
Etiology (N; %)					
ACEI/ARB	45 (37.5)	22 (18.8)	A+C	67 (28.3)	0.03
Idiopathic	32 (26.5)	31 (26.5)	98.4% A+C 1.6% FFP	63 (26.6)	
Insect byte	15 (13.5)	23 (19.7)	A+C	38 (16)	
Antibiotics; NSAID; OTC	11 (9)	17 (14.5)	A+C	28 (11.8)	
GERD	10 (8)	11 (9.4)	A+C+PPI	21 (8.9)	
Neoplasms	5 (4)	6 (5.1)	A+C	11 (4.6)	
HAE	2 (1.5)	7 (6)	specific	9 (3.8)	
Total (n)	120 (100)	117 (100)		237 (100)	

*Chi-square test; IQR=interquartile range; ACEI=angiotensin-converting enzyme inhibitors; ARB=angiotensin II receptor blockers; NSAID=NonSteroidal anti-inflammatory drugs; GERD=*G*astroesophageal reflux disease; HAE=Hereditary angioedema; N=Number of patients; A+C=Antihistamines+corticosteroids; FFP=Fresh frozen plasma; PPI=Proton pump inhibitor; OTC=Over the counter.

fully in only one patient in group A, when standard therapy failed. HAE patients were hospitalized for specific therapy, such as C1-INH concentrate (plasma derived or recombinant) or icatibant. In all patients presenting with mild AE (the majority of the population), monitoring in hospital for at least 6 h was advised. Patients with clinically significant obstruction of the upper airway (21%) were hospitalized in the intensive care unit or the Department of Otorhinolaryngology for a few days. There was no need for tracheostomy in any patient with ACEI-AE in either group. Tracheostomy was a life-saving treatment for one patient in group B with HAE.

Discussion

Recent studies have revealed AE as the most frequent and increasing disorder that results in hospitalization (14). In comparison with the study by Loftus et al., twice as many patients (21%) needed to be hospitalized for severe angioedema in our investigation (15). In 26.5% patients in group B the cause of AE was unidentified, which makes differential diagnosis and management even more challenging, especially in general hospitals with limited diagnostic and therapeutic tools. The role of GERD or Helicobacter pylori infection in AE remains unclear. Recent studies have presented the opposite results of AE resolution connected with eradication of Helicobacer pylori (10, 27-29). Patients indicating GERD angioedema etiology in this study (8.9%) were treated additionally with PPI. This is the first study in Croatia to analyze the frequency and treatment of bradykinin-induced angioedema as a major cause of emergency attendance. This investigation showed an etiological statistically significant difference between the groups (Chi-square, P=0.03) but the same therapeutic pattern in both clinical and general hospital centers. 20–45% of the patients admitted to the emergency rooms for suspicion of AE are suffering from an AE mediated by bradykinin (6, 15). However, reliable tests that can differentiate bradykinin angioedema from angioedema due to other causes are still not routinely available. In recent years,

the growth of the use of RAAS blockers has resulted in an increased prevalence of angioedema. The causes of AE listed in Table 1 show that ACEI/ARB was the main cause of emergency attendance in group A (37.5%) and among the leading causes in group B (18.8%). It occurs very often in patients who use several drugs for chronic therapy, such as diuretics (as in group A), accentuating the burden of ACEI among the at-risk population, especially in female patients, with well-known higher prevalence noticed in 70.5% females in our investigation. AE is characterized by a local, transient, asymmetrical, sudden and painful swelling of the subcutaneous (facial) and submucosal (oropharyngeal and laryngeal) tissues that occasionally requires tracheostomy as a life-saving treatment, as it did in one patient with HAE in our study (6). If ACEI is the unrecognized cause of angioedema, it relapses with ACEI therapy, as was the case in this study. The drugs commonly used to treat histamine-induced AE of allergic origin (glucocorticoids, antihistamines, epinephrine) are ineffective against bradykinin-induced AE (3, 6). In the investigated groups, ACEI-AE angioedema persisted for 2-4 days with poor resolution using these therapeutics. That emphasizes the need for new therapeutic solutions, especially when the patient's airway is compromised. Specific medications (plasma derived or recombinant C1-INH concentrate, icatibant) for HAE or acquired AE might be the treatment of choice, as has been demonstrated in numerous studies (21, 30-33).

There is a lack of studies using large cohorts of patients with ACEI-AE. The patients with ACEI-AE included in this study were not treated with C1-INH concentrate or icatibant, although in one patient with severe AE of the tongue, poor resolution of AE was noticed. However, the recent studies by Straka and Sinert et al. do not support the efficacy of a bradykinin B2 receptor antagonist in ACEI-AE (34, 35). Fresh frozen plasma that contains natural ACE and C1-INH effectively treats ACEI-AE, and was successfully applied in one patient with an unknown cause of AE (6). Plasma derived or recombinant C1-INH concentrate and icatibant were successfully administered during HAE attacks in all the investigated patients (3.8%) in groups A and B (36).

Conclusion

AE resulting from bradykinin-induced AE (ACEI/ARB and HAE) was the main cause of emergency attendance of patients. Identifying the cause and withdrawal of ACEI is key to management. Mild cases of ACEI-AE may respond to antihistamine or corticosteroid therapy, but moderate to severe cases do not. Advances in the treatment of HAE and case reports of patients with ACEI-AE treated with C1-INH concentrate or bradykinin receptor antagonist show that they may be a safe and efficacious therapeutic option for AE.

What is already known on this topic

Angioedema (AE) is a transient, localized swelling usually manifested in the upper airway, causing potentially life-threatening swelling of the mouth and throat. The most frequent mechanism of AE induced by drugs (10-25% of all cases, never associated with urticaria) includes inhibition of bradykinin degradation, induced by the renin-angiotensin-aldosterone system, such as angiotensin-converting enzyme inhibitors or angiotensin II receptor blockers. There is no approved treatment for this potentially life-threatening situation.

What this study adds

This is the first study in Croatia analyzing the frequency and treatment of bradykinin-induced angioedema as a cause of emergency attendance. Bradykinin-induced AE was the leading cause in the investigated group in a total of 75 (31.5%) patients. Our study confirmed the poor response to glucocorticoid, antihistamine and epinephrine treatment in severe AE, and the need for new therapeutic options to improve resolution of AE.

Authors' contributions: Conception and design: LjKL and IP; Acquisition, analysis and interpretation of data: LjKL, TP and JJZ; Drafting the article: LjKL and IP; Revising it critically for important intellectual content IP, PP and SD; Approved final version of the manuscript: LjKL, TP, PP, JJZ, SD, IP.

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Legal and Ethical Aspects of Pain Management

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In this manuscript we presented legal and ethical aspects of pain management. Pain is a global public health problem because the burden of acute and chronic pain is considerable and is continuously increasing. It has been postulated that pain management is a fundamental human right, and that health systems are obliged to ensure universal access to pain management services. The suggestion that pain management is a right was fuelled by ample evidence about inadequate treatment of pain. Undertreatment and underprescribing in the context of pain can potentially have serious legal consequences, including charges about negligence, elder abuse, manslaughter and euthanasia. Multiple international declarations by professional societies have outlined pain management as a core ethical duty in medicine. Therefore, healthcare professionals need to be aware of multiple facets of pain-related ethics, including appraisal of patient's decision-making capacity. The worldwide opioid crisis also calls for careful consideration of specific ethical issues. Finally, healthcare workers need to be aware of the the risks associated with promoting pain management as a human right because patients and their caretakers can mistakenly perceive that they have right to total analgesia. **Conclusion.** Patients do have the right to pain management, but patient rights have limits, which may interfere with other competing rights, and also rights of their physicians. Treatment of pain must be medically, ethically and economically justified. Healthcare workers have an obligation to continuously improve their knowledge about pain management, including medical, legal and ethical aspects of pain.

Introduction

The treatment of pain is a public health priority because the global burden of acute and chronic pain is considerable and it is continuously increasing (1). A recent analysis of the burden of chronic pain without clear etiology in individuals living in low and middle-income countries (LMIC) indicated that the prevalence of unspecified chronic pain is 34% in the general population living in LMICs, with 42% of the general population suffering from a headache, and 21% from low back pain (2). This analysis included 119 research reports from 28 LMICs, and the authors highlighted a high heterogeneity in results and assessment of global chronic pain (2).

The burden of chronic pain is pervasive not only in LMICs, but across the globe. This is supported by results of the Global Burden of Disease Study 2013, published in 2015, which analyzed global, regional and national incidence, prevalence and years lived with disability for as many as 301 diseases, both acute and chronic, and injuries in 188 countries between 1990 and 2013 (3). Their results indicated that

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among the top ten diseases/conditions that caused disability, low back pain was ranked at the first place, while chronic neck pain and migraine were ranked 4th and 6th, respectively. Two mental health conditions were also among the top 10, including major depressive disorder and anxiety (3); this is important in the context of pain because it is known that depression and anxiety are important comorbidities with chronic pain (4).

Chronic pain commands attention because it is usually difficult to treat, particularly certain types such as neuropathic pain (5). Currently, there is an ongoing debate whether chronic pain is a disease in its own right (6-9). However, acute pain is also a significant burden, despite myriad available treatment options for the management of such pain. Numerous recent studies have shown that acute pain is very prevalent across the globe, even when it is preventable. For example, a systematic review of acute pain in hospitalized patients, published in 2016, indicated that the prevalence of such pain ranged from 38 to 84%, while prevalence of severe pain ranged from 9 to36% (10). The manuscript analyzed 14 studies published from 1990 to 2013, included data from 9 countries, which involved a total of 23,523 patients from 56 hospitals (10).

Newer studies show similar data trends, indicating the high prevalence of pain in the hospital setting, lack of attention to available guidelines, and insufficient prescribing of pain medications (11-13). This is even found in the case of postoperative pain after major surgical procedures (14).

Furthermore, the socioeconomic impact of acute and chronic pain is severe. Pain is one of the leading reasons for absence from work in the USA, where lost productive time from common pain conditions among active workers translates into major costs (15). Gaskin and Richard have shown that the total incremental cost of healthcare that can be attributed to pain ranged from 261 to 300 billion USD. They valued lost productivity with three different estimates, including missed days of work (estimated at up to 12.7 billion USD), lost hours of work (up to 96.5 billion USD and lower wages (up to 225 billion USD). The total calculated cost of pain to society, including healthcare costs and all three productivity estimates, ranged from 560 to 635 billion USD in 2010 dollars (16). Based on the authors' results, the annual cost of pain in the USA was higher than the annual cost of heart disease (estimated at 309 billion USD), cancer (243 billion USD) or diabetes (188 billion USD) (16).

It needs to be emphasized that while attention to pain has increased in the international research community, the attention is shifting to different topics. For example, analysis of manuscripts published in a prominent pain-related journal from 1975 to 2007 indicated that the percentage of studies analyzing interventions in humans declined precipitously with time as a percentage of publications, replaced with an increased number of animal studies about behavioral pharmacology (17). Furthermore, it was recently emphasized that attention to pain is swinging between extremes and represents a moving target, with most of the articles in lay press highlighting the problems associated with opioid analgesics, while neglecting significant benefits that can be accrued from their appropriate use (18). There are still areas of the world where the use of opioids is insufficient considering the estimated burden of disease (19).

The aim of this manuscript was to present legal and ethical aspects of pain management, with regards to relevant references and new developments in the field of pain.

Pain management is a fundamental human right

It has been postulated that pain management is a fundamental human right, and

that health systems are obliged to ensure universal access to pain management services on a nondiscriminatory basis including provision of basic medications for treatment of pain and implementation of national pain policies (20,21).

After recognizing that pain management is a human right, the next challenge is to ensure implementation of appropriate medical and social changes that will make pain management a core component of healthcare (22). Pain management is a core ethical duty in medicine (20). Frustrated by the slow pace of medical, cultural, legal, and political change, many within the community of pain clinicians have begun to promote the status of pain management beyond that of appropriate clinical practice or even an ethic of good medicine (20).

Law and management of pain: Negligence, elderly abuse, euthanasia

In common law, the suggestion of negligence provides a further possible legal foundation that should ensure adequate pain management. Margaret Somerville, Professor of Law and Medicine at McGill University, has long argued that the unreasonable failure to provide adequate pain management constitutes negligence (23). The law of medical negligence emphasizes taking reasonable care in all aspects of patient management. When approaching a physician, patients expect medical treatment that will relieve their medical problems. A physician owes certain duties to patients and breach of these duties may give a cause of action for negligence against a physician (24). Undermedication of pain has also been called a moral negligence (25).

With respect to pain control, doctors may breach their standard of care by failing to take an adequate pain history from the patient; by treating the pain inadequately; and, in the context of uncontrolled pain, by failing to consult an expert in pain management (26). Several such cases of legal actions involving pain-related negligence have already appeared; they have even been described in medical literature, reporting that millions in damages were paid because of gross negligence associated with undertreatment of pain and suffering (27). Ben A. Rich has warned physicians: "Another message to physicians implicit in these verdicts is that there is a standard of care for pain management, a significant departure from which constitutes not merely malpractice but gross negligence. Even if professional boards might not hold their licensees to that standard, juries will" (27).

One of those cases indicates that geriatric setting is yet another area of jurisprudence that may be associated with legal aspects of pain management (27, 28). In a case of Bergman vs Chin it was argued that according to California's Elder Abuse and Dependent Adult Civil Protection Act, inadequate pain management of an elderly person may be considered a case of elderly abuse (28).

Pain management of terminal patients is yet another potential legal problem because some pain medications can be life threatening. Severe adverse events, including deaths, are the most commonly reported adverse effects of opioids (29). It has been argued that giving pain medication to terminal patients could be mistaken for a manslaughter and a sort of euthanasia (30). This legal problem for physicians could be prevented by consulting physicians specialized in pain management such as those employed in pain clinics (31). Furthermore, physicians need to take due care to avoid such risk of being liable, including taking an informed consent for treatment, observing a proportionality rule, and accurate keeping of medical records (30). With time, more cases may emerge to better outline the boundaries of reasonable action by doctors, nurses, and pharmacists in pain management (26).

Ethics and management of pain

The management of pain has been postulated as a "core ethical duty in medicine" (22). The responsibilities of doctors to their patients are primarily ethical. Right to a pain management could be considered as an example of a bioethical principle of beneficence, or doing good for others, which is an ethical principle that is particularly prominent in medicine (32). This principle was ingrained in a number of key documents and declarations adopted by relevant international medical organizations.

According to the Americal Medical Association (AMA)'s Code of Ethics, 'physicians have an obligation to relieve pain and suffering" (33). Since 1947, the World Medical Association (WMA) has been developing a system of ethical obligations or a professional deontology, which elaborates the physician's role versus the rights of individual patients. In 1948, the WMA published a Declaration of Geneva, which was proclaimed to be "the contemporary successor to the 2500-year-old Hippocratic Oath" (34). One of the principles of the Declaration of Geneva is "the health and well-being of my patient will be my first consideration" (34). Professional associations of healthcare personnel in many countries have adopted a similar ethical basis for the management of pain (20). The WMA has also brought forward the International Code of Medical Ethics in 1949, last updated in 2006 (35). Both in this Code and in Declaration of Geneva, patient rights are presented as the result of the matrix of obligations of a physician towards a patient (34,35).

In 1964, the WMA adopted a Declaration of Helsinki (DoH) as the first international document prescribing rules for medical research involving human subjects (36). The purpose of DoH was to provide guidance to those involved in clinical research, and therefore its main focus was a responsibility of a researcher regarding protection of humans as research subjects. The DoH defined requirement to obtain informed consent of participants, as a measure for protecting an individual's autonomy. Initially this was meant as a protection against unwanted inclusion in experimentations, but informed consent later became generally accepted standard for consenting to a medical treatment, as specified in the WMA's Declaration of Lisbon on the rights of the patient, which was first adopted in 1981 (37).

Together with the bioethical principle of beneficence, the principle of nonmaleficence is also crucial in medicine, which prohibits the infliction of harm (38). Failure to reasonably treat patients' pain and suffering causes harm could, therefore, be considered an ethical breach of maleficence (39).

The American Academy of Pain Medicine (AAPM) has therefore adopted the ethics charter, which requires all physicians to improve in a number of pain-related areas, including assessment of pain, treatment of pain with competence and compassion, education in principles of pain medicine, support to pain-related research and engagement in advocacy that will ensure access to pain management and its continuous improvement (40).

International Association for the Study of Pain (IASP) also took a stand on the issue of ethics and pain, by adopting a Declaration of Montreal during the First International Pain Summit on September 3, 2010, which states that access to pain management is a fundamental human right (41). IASP was founded in 1973 and it is the largest multidisciplinary international organization in the field of pain, which "brings together scientists, clinicians, health-care providers, and policymakers to stimulate and support the study of pain and translate that knowledge into improved pain relief worldwide" (42). As the foremost global organization devoted to pain research and management,

IASP has endorsed numerous ethical principles and declarations about including humans in research endeavours (43).

Decision-making capacity in patients with pain: Ethical and legal issue

Physicians who treat patients suffering from pain need to be aware about their patients' decision-making capacity. Pain can also be a factor that negatively affects decision-making capacity. Iatrogenic causes can contribute to diminished patient's decision-making capacity. Therefore, physicians treating pain have an ethical and legal obligation to assess and evaluate patient's decision-making capacities (40). Assessing whether a patient has capacity to make informed decisions about his or her medical care is something that physicians are frequently required to do (44). According to the AAPM, giving autonomous informed consent requires from a patient: i) understanding of information and consequences, ii) demonstration of insight, iii) reason and judgment, iv) the ability to evince a decision or articulate a preference and v) voluntariness (40).

Physician needs to respect patient's wishes and values. All five criteria must be met for informed decision-making. If a patient cannot meet these criteria, a surrogate decision-maker needs to be involved. Alternatively, autonomy of patients with diminished or impaired decision-making capacity can be promoted by giving them degree of autonomy commensurate with their capabilities (45).

Sometimes, a physician may perceive that a surrogate decision-maker is not suggesting actions that are in the patient's best interest. Ethical and legal obligation of a physician is to advance welfare of a patient. If necessary, an attending physician may consult institutional ethics committee to seek help regarding medical decisions. Ethics committee needs to be the first instance to refer such a dispute before resorting to legal actions (45).

Ethics of opioid prescribing for pain management

Use of opioids for pain management has recently emerged as a particular ethical problem, particularly in the setting of treating non-malignant chronic pain (46, 47). Overprescription and overmarketing of opioids in certain areas such as North America and Australia and has lead to "opioid crisis", and science is called upon to resolve this public health threat (48). Although opioids are effective painkillers, their side effects necessitate a careful approach in chronic non-cancer pain states (49).

In USA, the number of opioid prescriptions written in 1998 was 100 million, increasing to 190 million prescriptions in 1998 and 290 million prescriptions in 2017. Consequently, more then ten million USA citizens are using prescription opioids for non-medical reasons, and about 2 million people were diagnosed with opioid addiction (47). According to the Centers for Disease Prevention (CDC), more than 33,000 Americans died from an opioid overdose in 2015, a number similar to the 35,000 and 36,000 deaths that were attributed to motor vehicle accidents and firearms in the same year (50).

These figures are a call to action and CDC has issued a guideline for primary care physicians with the aim to reduce opioid prescriptions for chronic pain except in cases of cancer treatment, palliative care and end of life care (51). This guideline is supported by weak evidence about efficacy of opioids for long-term management of chronic non-cancer pain (52). Furthermore, systematic review that analyzed randomized controlled trials and observational studies about effectiveness and risks of long-term opioid therapy for chronic pain was recently published (53). After searching the literature, the authors reported that they could find „no study of opioid therapy versus no opioid therapy evaluated long-term (>1

year) outcomes related to pain, function, quality of life, opioid abuse, or addiction", and therefore there is "insufficient evidence to determine the effectiveness of long-term opioid therapy for improving chronic pain and function" (53).

Based on these data, Erdek and Pronovost have suggested that the dilemma regarding overprescribing and underprescribing of opioids requires "a balanced approach based on an ethical framework; policy changes will need to be implemented in order to effect a more rational approach to the use of opioids; and as with any change, the new policies will likely defend against some risks and introduce new ones, often requiring iterative policy changes" (47).

Policy changes that have been introduced to halt opioid crisis appear to be effective. Drug monitoring programs introduced in USA were associated with lower opioid-related mortality rates (54). Particularly states where these drug monitoring programs had robust characteristics, such as monitoring higher numbers of drugs with abuse potential and updating their data at least weekly, had greater reductions in deaths, compared to other states whose drug monitoring programs did not have these characteristics (54).

If opioid therapy has to be prescribied for chronic noncancer pain, physicians need to schedule frequent follow-up visit to assess improvement and perform urine drug testing to monitor patient compliance (47). Furthermore, physicians need to engage in open discussions with patients and their families about benefits and harms of opioid therapy. Healthcare systems and all its relevant stakeholders have an obligation to optimize patient satisfaction, while minimizing harms to individuals suffering from chronic pain and to society at large (47). Countries in different continents have started responding to opioid epidemic with prescribing guidelines that need to ensure that opioids will be prescribed for appropriate indications only, in limited doses, for carefully selected patients. Additionally, patients need to be advised on safe use of opioids (55). Pain community has reached a broad consensus that opioid epidemics that we are witnessing in certain countries need to be addressed urgently and that other countries should be protected from similar negative outcomes (55).

The problems with opioid crisis may not generalize to the rest of the world; hopefully there are mechanisms in place in each country to prevent this. This also depends on health care resources of each country. The opioid crisis in the USA is a consequence of direct to consumer advertising, untruthful marketing of opioids to physicians and the lack of physician education about pain and its treatment. Each of these factors needs to be studied by every country to make sure that they do not replicate these mistakes. Ultimately, it could be argued that pain education in curricula of healthcare professionals needs due attention.

Precariousness of promoting pain management as a right

We now have sufficient evidence about inadequate treatment of pain that we can talk about "ethics of undertreatment" and "ethics of underprescribing" (26). However, we also see now how opioid crisis is raising additional set of ethical questions. Thus, promoting pain management as a human right as a legal right may lead to patients and public to request any analgesic they want. The erroneous interpretation of "right to pain relief" may potentially disrupt the basic tenets of clinical assessments by physicians and other healthcare workers (26).

Likewise, it is important to balance the message regarding pain management as a right because not all types of pain can be adequately treated (56). Therefore, while promoting pain management as a human right, we have to be careful to also convey

the message that "pain relief is not the right to a pain-free life" as there is no guarantee of perfection in medicine (26). If pain management as a human right is interpreted as a right to total analgesia, this will easily lead to frustration among patients and their carers and potentially to litigation (56). Therefore, whenever we talk about pain management as a human right, healthcare professionals need to make clear that this right implies "reasonable and proportionate" response to the intensity and type of pain a person is experiencing (26).

On the other side, it has been argued that promoting pain management as human right can also have negative unintended consequences among healthcare professionals who may see such movements as a potential threat to their clinical judgment and autonomy, and in response become more rigid and defensive, ignoring the clinical guidelines and recommendations for best practices (26).

Thus, education is crucial, both of patients and their carers, as that of healthcare workers. Patients and their carers need to be educated by their attending healthcare staff in what they can realistically expect from the current state of medical science. For education of healthcare workers we need institutional acceptance of pain-related curricula on all levels of professional education. In this respect, IASP made significant advances towards improving professional training about pain by publishing multiple curricula, which cover both core curriculum about pain education and discipline-specific curricula. These curricula are intended to help in establishing teaching courses on acute, chronic and cancer pain at various educational levels, including both undergraduate and graduate level. All these curricula are freely available online, and they were last updated in May 2012. These curricula include medical, legal and ethical aspects related to pain assessment and treatment (57).

Conclusion

Pain management is fundamental to good clinical practice. Patients do have the right to pain management, but also patient rights have limits, which may be limited by other competing rights, and also rights of their physicians. Treatment of pain must be medically, ethically and economically justified. Attending physician always needs to evaluate a patient thoroughly, and recommend evidence-based treatment for pain, while educating patients about what realistically can be expected from the treatment. Healthcare workers have an obligation to continuously improve their knowledge about pain management, including medical, legal and ethical aspects related to pain.

What is already known on this topic

For almost two decades now the pain management has been promoted as a fundamental human right. Legal issues and ethical declarations related to pain have also been accumulating, as well as elaborations about precarious issue of pain management, particularly in sensitive cases such as care for terminally ill patients. Major international organizations realized that they need to devote particular attention to ethics and legal aspects of pain.

What this study adds

In this review we summarized the main legal and ethical aspects of pain management, with particular focus on relevant international declarations and ethical codes and specific cases that physicians should be aware of. Underprescribing and overprescribing of pain therapies can be associated with accusations of negligence, elderly abuse and euthanasia attempts. We also addressed novel controversies related to ethics of the current opioid crisis, perils of promoting pain management as a human right, and pointed out what major international organization for the study of pain recommends as curricula for adequate education of healthcare workers about pain management.

Authors' contributions: Conception and design: MJ; Acquisition, analysis and interpretation of data: MJ and LP; Drafting the article: MJ and LP; Revising it critically for important intellectual content: MJ and LP; Approved final version of the manuscript: MJ and LP.

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Early Machine-Human Interface around Sepsis Severity Identification: From Diagnosis to Improved Management?

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Objective. To investigate the statistical measures of the performance of 2 interventions: a) early sepsis identification by a computerized sepsis "sniffer" algorithm (CSSA) in the emergency department (ED) and b) human decision to activate a multidisciplinary early resuscitation sepsis and shock response team (SSRT). **Methods.** This study used a prospective and historical cohort study design to evaluate the performance of two interventions. **Intervention.** A computerized sepsis sniffer algorithm (CSSA) to aid in early diagnosis and a multidisciplinary sepsis and shock response team (SSRT) to improve patient care by increasing compliance with Surviving Sepsis Campaign (SSC) bundles. **Results.** The CSSA yielded a sensitivity of 100% (95% CI, 99.13-100%) and a specificity of 96.2% (95% CI, 95.55-96.45%) to identifying sepsis in the ED (Table 1). The SSRT resource was activated appropriately in 34.1% (86/252) of patients meeting severe sepsis or septic shock criteria; the SSRT was inappropriately activated only three times in sepsis-only patients. In 53% (134/252) of cases meeting criteria for SSRT activation, the critical care team was consulted as opposed to activating the SSRT resource. **Conclusion:** Our two-step machine-human interface approach to patients with sepsis utilized an outstandingly sensitive and specific electronic tool followed by more specific human decision-making.

Introduction

Approximately 750,000 patients develop severe sepsis and septic shock in the United States each year (1). More than half of them are admitted to an intensive care unit (ICU) accounting for 51% of all ICU admissions, 20-30% of hospital deaths, and $15.4 billion in annual health care costs (1-3) Early diagnosis and treatment of these patients is critical and is associated not only with improved morbidity and mortality, but also reduced length of stay (LOS) (4-7). After the landmark randomized control trial by Rivers et al (2001) endorsing early goal directed therapy (EGDT), institutions around the world have developed, implemented and revised quality improvement (QI) initiatives to incorporate EGDT into clinical practice (4).

The delayed diagnosis of sepsis coupled with the lack of reliable rule-out criteria in the emergency department (ED) has been associated with high mortality risk, which dictates improvements in the timeliness of sepsis detection by developing an automated system (8-11). The available published literature regarding systems within Electronic

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health records (EHR) to improve early identification of septic patients substantially differed in triggering criteria, effector responses, and study settings. Results of the studies investigating the effect of computerized monitoring in addition to response in the non-ICU acute inpatient setting endorsed an improvement in the timeliness of diagnostic and therapeutic interventions (11), but have not significantly affected patient outcomes (8). This may be due to inadequacies in triggering criteria or inadequacies in response to the alerts. Overall, the reported sensitivity and specificity in previous studies on computerized algorithms for sepsis identification remained unsatisfactory (12, 13). The team approach to sepsis care has proven effective as evidenced by improved bundle compliance and patient outcomes. A prospective study analyzed outcomes following implementation of standardized interdisciplinary order sets for patients with severe sepsis/septic shock. Sepsis order sets implemented using the Six-Source Influencer Model to create an atmosphere of teamwork to help counteract resistance to change, improved the process of care (14). A more recent study published in 2016 evaluated the effect of a QI sepsis management bundle on mortality and sepsis protocol compliance, determining that interdisciplinary ED sepsis bundles composed of triage flagging, registered nurse (RN) medical directives, education campaigns, and modified sepsis protocols improved early diagnosis and protocolized medical care resulting in improve care (15).

In line with the literature, Mayo Clinic Florida (MCF) employed multiple measures to improve the care of the septic patient, specifically in the emergency department (ED). A computerized sepsis sniffer algorithm (CSSA) to aid in early diagnosis and a multidisciplinary sepsis and shock response team (SSRT) to improve patient care by increasing compliance with the Society of Critical Care Medicine (SCCM) bundles was deployed in September 2013 as part of a QI project already in place (16). Examination of these QI efforts over a period of three months revealed a low observed-to-expected incidence of sepsis, prompting this QI effort. MCF's CSSA was revamped multiple times, aiming to find the appropriate degrees of sensitivity and specificity. While there is research on computerized algorithms to diagnose sepsis and the team approach to the septic patient, there is a dearth of quantitative research related to the human response to these methods.

Therefore, the purpose of this study was to: (1) evaluate the effectiveness of the CSSA, and (2) evaluate the human decision to activate the SSRT once computerized algorithms recognize a patient meeting sepsis criteria.

Methods

The cyber-realm of caring for the septic patient at MCF starts when a computer algorithm recognizes a patient with systemic inflammatory response syndrome (SIRS) or sepsis based upon qualifying criteria. Once triggered, the computer algorithm alerts the ED team lead nurse who notifies the ED provider that the patient met criteria for either SIRS or sepsis. The ED physician then evaluates the patient and confirms whether the patient is actually septic. The ED nursing staff utilizes a checklist to promote completion of all bundle requirements.

In addition to the computer algorithm, MCF also developed a multidisciplinary Sepsis and Shock Response Team (SSRT) composed of an ICU physician, an ICU fellow or resident, an advanced practice provider (APP), a nursing supervisor, and a pharmacist. Once the ED provider confirms the patient has either severe sepsis or septic shock, the SSRT is activated and the team is expected to report to the ED within

15 minutes to evaluate the patient, encourage completion of bundle requirements, and collaborate with ED providers to triage the patient to the appropriate level of inpatient care. The SSRT is not to be activated for sepsis only patients. Using multiple Plan-Do-Study-Act (PDSA) cycles, the QI team at MCF tracks bundle compliance, provides feedback to providers at monthly staff meetings, and communicates feedback via bi-weekly e-mails.

Study design

This study used a prospective and historical cohort study design to evaluate the performance of two interventions. The study proposal was approved by the Mayo Clinic Institutional Review Board (IRB) as a QI project. Review of the electronic medical record (EMR) was done with pre-obtained patient consent.

Study population

To have an accurate assessment of the CSSA, our study included all ED patients who had sepsis sniffer alerts and SSRT activations with a final principal diagnosis of septicemia resulting in a diagnosis-related group (DRG) of septicemia or severe sepsis (MS-DRGs 870-872) during the study period September 1st 2013 to August 31st 2014.

Data collection

Based on discharge DRGs of septicemia or severe sepsis, physician reviewers retrospectively abstracted patient demographic information, Acute Physiology and Chronic Health Evaluation (APACHE) IV scores, rates of ICU consultations, hospital length of stay (LOS), and mortality. Two physician reviewers (V. B. and M. M.) categorized patients based on standardized clinical definitions of sepsis, severe sepsis and septic shock, approved by Mayo Clinic enterprise subspecialty councils for the Departments of Emergency Medicine and Critical Care Medicine (Appendix 2). Reviewers were blinded to whether or not the CSSA was triggered or the SSRT was activated. Reviewers evaluated the cohort over two distinct time periods: the first four hours after ED presentation (0-4 hours) and the subsequent 8 hours (4-12 hours) in order to assess if after initial management and triage, septic patients progressed to a more severe end of the sepsis spectrum and required escalation of care.

Three ED nurses (N.D.,A.R., and E.G.), part of a larger continuous QI initiative already in place, incorporated data on CSSA triggers and subsequent SSRT utilization in to the historic cohort based on DRGs. Additionally two ED nurses (N.D & A.R.) retrospectively provided documented code status on admission.

Statistical analysis

Outcomes and variables were assessed using descriptive statistics. Continuous variables were reported using medians with the 1st and 3rd interquartile range (IQR). Sensitivity, specificity, false positive rate were calculated for the CSSA triggers. Sepsis and Shock Response Team utilization was reported as "appropriate" or "inappropriate" utilization. We first performed univariate analyses to determine the unadjusted associations of the SSRT activation and other predictor variables with the mortality due to sepsis on hospital discharge. We used nonparametric tests (Fisher's and Wilcoxon, as applicable) assuming the worst case scenario of non-normalcy. A P value of less than 0.2 was considered statistically significant. Inclusion decision for P-value of 0.2 could be justified by the exploratory character of the multivariate analysis and by our intention to fare on the more conservative side. We excluded

age from the multivariate logistic regression because of inclusion of APACHE-IV Score. The risk estimates were reported as odds ratios (OR) with 95% confidence intervals (CI). The data was analyzed using the JMP® Pro 10.0.0 statistical software package (SAS Inc., Cary, NC, USA).

Results

A total of 27,106 patients presented to the ED between September 2013 and August 2014. A total of 1,431 patients triggered the CSSA within four hours of arrival to the ED. Of those CSSA triggers, 424 patients were deemed septic based on discharge DRGs. Upon manual review of the 424 patients, 419 were truly septic within 4 hours of presentation to the ED. Therefore, CSSA yielded a sensitivity of 100% (95% CI, 99.12- 100.00%) and a specificity of 96.21% (95% CI, 95.97 -96.43%) in identifying sepsis in the ED (Table 1). Thirty-four percent of the 252 patients meeting criteria for severe sepsis or septic shock, as determined by retrospective review, had an appropriate SSRT activation. In 53% (134/252) of cases meeting criteria for SSRT activation, the ICU was consulted directly as opposed to first activating the SSRT resource (Figure 1, Table 2 and Online supplemental material). The SSRT was inappropriately utilized only three times in sepsis-only patients.

In the initial phase (0-4 hours), 167 patients met criteria for sepsis-only, 176 met criteria for severe sepsis, and 76 met criteria for septic shock. Five patients were deemed not septic (Figure 1). In the second time period (hours 4-12), one patient progressed from sepsis-only to severe sepsis and two patients progressed from severe sepsis to septic shock. An ICU consult was obtained in one of two patients with septic shock. Of the five patients who did not meet sepsis criteria during the first four hours, over the following 8 hours (hours 5-12) one developed sepsis-only, two developed severe sepsis, one developed septic shock, and one remained sepsis-negative (Online supplemental material).

In univariate analysis, APACHE IV, full code status on admission, SSRT activation and ICU consultation were variables associated with in-hospital survival (Table 3a). In

Table 1. Screening performance characteristics for computerized sepsis sniffer algorithm in ED patients

Sepsis sniffer performance	Sepsis	No sepsis	Total	Sensitivity	Specificity	FP rate
Activation	419	1012	1431	100.00% (95% CI: 99.12- 100.00%)	96.21% (95% CI: 95.97 -96.43%)	3.79%
No activation	0	25675	25675			
Totals	419	26687	27106			

ED=Emergency department; CI=Confidence interval; FP=False positive rate.

Table 2. Performance characteristics for human interface decision in severe sepsis or septic shock ED patients in the first 4 hours

Intervention	Severe sepsis or septic shock (n=252)	Sepsis only (n=167)	Total (n=419)	Appropriate utilization	Inappropriate utilization
SSRT activation	86	3	89	34.1% (86/252)	1.8% (3/167)
No SSRT activation	166	164	330		
ICU consult	134	19	153	53.2% (134/252)	11.4% (19/167)
No ICU Consult	118	148	266		

ED=Emergency department; SSRT=Sepsis and shock response team; ICU=Intensive care unit.

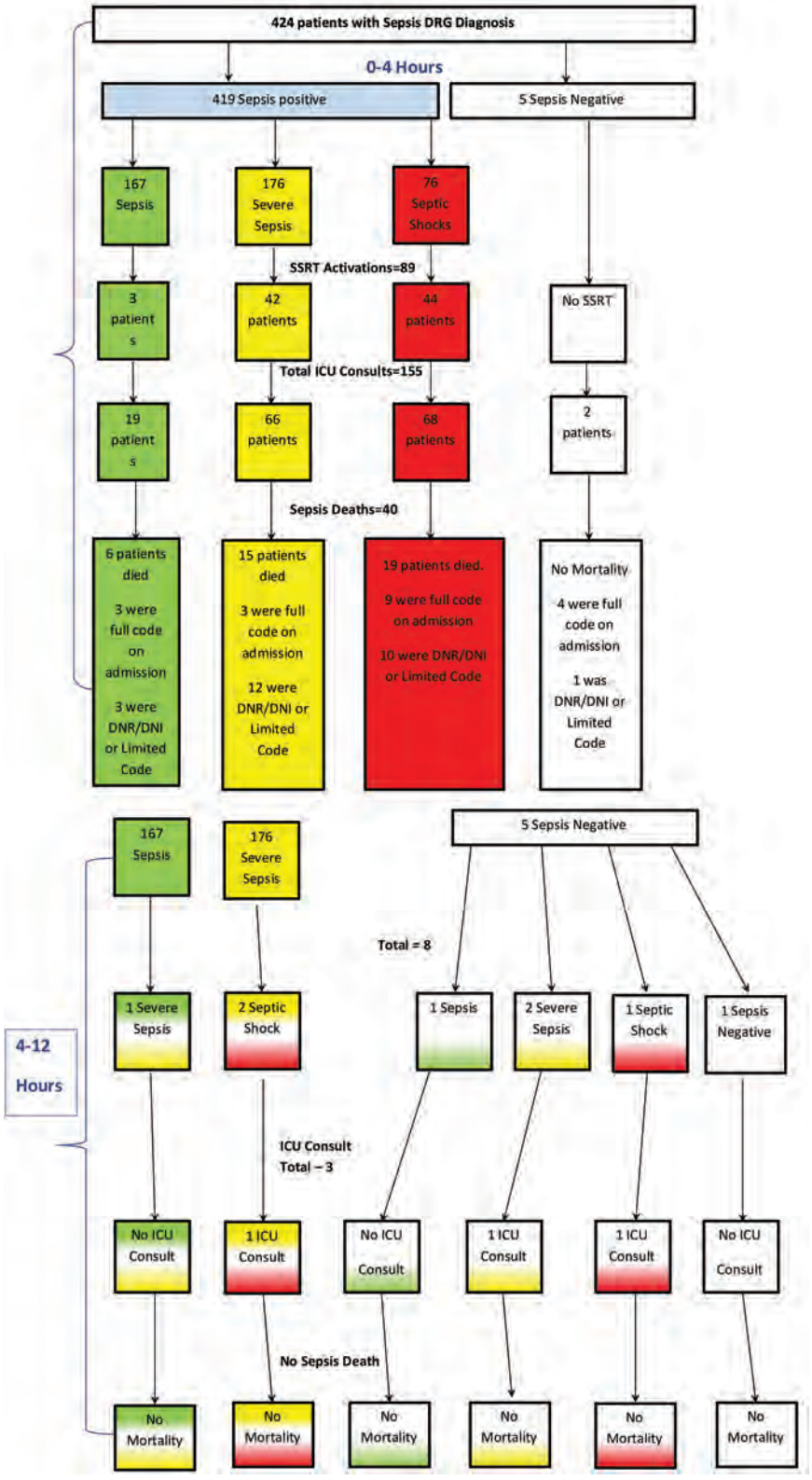

Figure 1. Study Flow chart for Patients Who Were Admitted between September 2013 and August 2014 and had a Sepsis Diagnosis Related Group Code Present upon Discharge, Mayo Clinic Florida.

Table 3a. Univariate analysis of the whole cohort

Patient characteristics	Alive	Dead	Odds ratio	P value§
Number of patients (%)	384/424 (90.6)	40/424 (9.4)	-	-
Age, median, IQR	67.5 (55-79)	75 (63-89)	0.96 (0.94-0.99)	<0.01
Male sex (%)	196/219 (89.5)	23/219 (10.5)	0.77 (0.40-1.49)	0.51
APACHE IV, median, IQR	84 (72-95)	97 (85.5-112.5)	0.96 (0.95-0.98)	<0.01
Full code status (%)	317/332 (95.5)	15/332 (4.5)	7.89 (3.95-15.76)	<0.01
SSRT activation (%)	73/89 (82)	16/89 (18)	0.35 (0.18-0.70)	<0.01
ICU Consult (%)	125/155 (80.6)	30/155 (19.4)	0.16 (0.08-0.34)	<0.01

Table 3b. Univariate analysis for severe sepsis and septic shock cases only

Patient Characteristics	Alive	Dead	Odds ratio	P value§
Number of patients (%)	218/252 (86.5)	34/252 (13.5)	-	-
Age, median (IQR)	68 (55.8-81)	74 (63-89.3)	0.97 (0.95-0.99)	0.02
Male sex (%)	116/135 (85.9)	19/135 (14.1)	0.90 (0.43-1.86)	0.85
APACHE IV, median (IQR)	86 (75-100)	98.5 (84.8-115)	0.97 (0.95-0.99)	<0.01
Full code status (%)	173/185 (93.5)	12/185 (6.5)	7.05 (3.24-15.32)	<0.01
SSRT activation (%)	70/86 (81.4)	16/86 (18.6)	0.53 (0.26-1.11)	0.12
ICU Consult (%)	107/134 (79.9)	27/134 (20.1)	0.25 (0.10-0.60)	<0.01

ICU=Intensive care unit; IQR=Interquartile range; SSRT=Sepsis and shock response team.
§ Nonparametric tests (Fisher's and Wilcoxon, as applicable).

Table 4a. Multivariate logistic regression analysis for the whole cohort

Mortality	Odds ratio	P value
APACHE IV	0.98 (0.96-0.99)	0.01
Full code status	7.71 (3.65-16.26)	<0.01
SSRT activation	0.69 (0.31-1.56)	0.38
ICU consult	0.22 (0.09-0.54)	<0.01

Table 4b. Multivariate logistic regression analysis for severe sepsis and septic shock cases only

Mortality	Odds ratio	P value
APACHE IV	0.98 (0.96-0.99)	0.03
Full code status	7.33 (3.21-16.74)	<0.01
ICU consult	0.26 (0.10-0.67)	<0.01

multivariate analysis; full code status (OR 7.71; 95% CI 3.65-16.26; P value <0.01*) and ICU consultation on admission (OR 0.22; 95% CI 0.09-0.54; P value <0.01*) retained significant association with survival, while SSRT activation consultation did not (OR 0.69; 95% CI 0.31-1.56; P value 0.38) (Table 4a). In subgroup analysis of patients with severe sepsis/septic shock, SSRT activation did not show a statistically significant association with survival in univariate (OR 0.53; 95% CI 0.26-1.11; P value 0.12) (Tables 3b and 4b).

The total combined in-hospital mortality of patients with sepsis, severe sepsis, or septic shock was 9.4% (40/424). Of note, 62.5% (25/40) of expired patients had active Do Not Resuscitate (DNR)/Do Not Intubate (DNI), or limited code at admission (online supplemental material).

Discussion

This study reports on our academic medical center's experience with the development and implementation of a 2-step machine-human interface approach to the septic patient in ED, including the CSSA (machine

SIRS ALERT: Any 2 of the following triggers yellow exclamation point which makes the patient level 2 and team leader paged: Temp=< 36C (96.8F), =>38.C (100.4F) oral, => 39C (102.2F) core SBP <= 90 RR >24 HR >100 MAP <=65
SEPSIS ALERT, a second red exclamation point alert would trigger and the team leader paged, sepsis checklist would be started and clinician asked to evaluate for signs of severe sepsis and activate the team (SIRS alert PLUS one of the following) WBC >=12K or <=4K Blood cultures ordered OR Lactate >4 alone
Keep both sniffers "on" for 2 hours post admit

Figure 2. Computerized Sepsis "Sniffer" Algorithm (CSSA)
Algorithm characteristics and cut-off point were derived, tested and refined by the authors at Mayo Clinic Florida in 2013.

algorithm, Figure 2) and the ED physician decision to activate SSRT (human interface).

Evidence suggests that early diagnosis, timely initiation of appropriate antibiotics, and resuscitation to hemodynamic goals, improve clinical outcomes (4, 6, 14, 17-23), while a multidisciplinary approach has been promoted in practice-based guidelines for sepsis management (24). The inability to transform evidence based medicine into clinical implementation has been recognized as one of the great obstacles of modern medicine. Irrespective of improvements in the proper care of critically ill patients, evidence-based interventions continue to be underused (25-29). The barriers consist of resource under-utilization for the critically ill in EDs, poor communication, poor transitions of care, and resistance to multidisciplinary coordination of care. Hewett et al (30, 31) have also proposed that the inter-professional and intergroup climate in hospitals leads to poor communication and impedes system approaches to improving healthcare. An intergroup environment in the healthcare facility represents persons identifying with their professional groups, and afterwards, valuing their groups over others, resulting in very poor interaction, unhealthy cooperation, competition for limited resources, and impedance of system standards (31). Types of intergroup members who might be linked to sepsis consist of ED physicians, intensivists, ED nurses, critical care nurses, pharmacists, medical informatics professionals, and hospital administrators. Barriers to implement this project include both the areas of clinical informatics, human factors and implementation science. From the clinical informatics perspective our current EMR does not have the capabilities to send automatic alerts, therefore we have to rely on including another layer of clinical informatics to get the data out of the EMR and run the CSSA algorithms requiring several validation tests. From the human factors and implementation science a significant hurdle we had to overcome was the silos type practice each group had in the ED, ICU and hospital service. By developing standardized tools, protocolized ways to communicate and respond depending on sepsis severity were key to overcome these barriers. This project provided a structured setting during which all parties involved in the care of these complex patients could sit together to actively identify and remove barriers to successful implementation of the bundle. Prior to the initiation of the project, the ED and ICU teams were working in silos and each had their own opinion as to who was responsible for which elements of the

bundle and even which patients required ICU level care. We were able to eliminate this ingrained hospital intergroup tradition by identifying its presence and developing a multidisciplinary SSRT that incorporated team members from almost all key groups coupled with the assignment of respected champions to modify the culture within each individual group. One of the keys to the success of this attempt was to spend time on the front end of the project carefully analyzing key stakeholders and their positions on the elements of the bundle and then painstakingly creating a cohesive team who could agree on triggers for the pager and SSRT activation and, more importantly, create consensus and a spirit of cooperation and teamwork within their respective groups. The creation of the SSRT in particular required buy-in from multiple members of the healthcare team. This strategy enhanced communication and resulted in consensus towards responsibilities among disciplines, where before, obstacles had hindered the administration of treatment protocols.

Utilizing clinical decision support (CDS) systems within electronic health records (EHRs) to improve early identification of septic patients has also been proven beneficial (32-34). Different CDS tools, like the Sepsis Early Alert Tool (SEAT) (32) and the Severe Sepsis Best Practice Alert (SS-BPA) (34) have been evaluated and proven effective as evidenced by decreased time to antibiotic administration in septic patients. The SEAT decreased median time from triage to antibiotic administration by 20 minutes (32). Also, the SS-BPA, an automated, real time surveillance system for patients meeting sepsis criteria within the EHR, was associated with a higher proportion of patients receiving antibiotics within one hour of diagnosis (34). One hospital sought to develop a sepsis surveillance system that was highly sensitive and specific by initiating an alert only after assessing the patient demographics, vital signs, medications, lab values, discrete documentation elements, medical problems, infectious diagnoses, and signs and symptoms of infection. The novel sepsis screening tool, taking into account an array of factors, reported a sensitivity of 95% and specificity of 82% when compared to the gold standard (physician chart review) (33). Despite the fact that current sepsis identification and alert systems have emphasized addressing clinical outcomes, these types of designs have not been successful in providing evidence of benefits in clinically meaningful or patient-centered endpoints (11, 12, 31, 32). Therefore, a better strategy is required to develop and validate a clinically beneficial sepsis alert system, particularly for performance in the ED and critical care environments. Our strategy focused on early recognition through CSSA, which was applicable to all patients presenting to the ED with sepsis. Although our alert system included the same variables as in the study by Brown et al (35), we achieved higher sensitivity with a lower false positive rate (100% and 4% versus 80% and 7 %, respectively). A reason for the lower false positive rate compared to Brown et al (35) could be that we adjusted the traditional systemic inflammatory response syndrome alert for respiratory rate and heart rate to 24 breaths/minute and 100 beats/minute, respectively (Appendix 1) to avoid alert fatigue. Our results are also comparable to the results of Manaktala et al (33), with the notable difference that they focused on electronic surveillance of the regular ward patients.

A major goal of this study was to improve the human interface, which was defined as the ED providers' decision to activate the SSRT after the machine interface identified a patient as potentially septic. Based on laboratory values and vital sign criteria, our CSSA was highly sensitive to diagnosing sepsis. Due to lack of advanced algorithms to detect severe sepsis; we rely on human

factor as the main driver of identification of severe sepsis and decision to activate SSRT. Based on human decision, the SSRT was appropriately utilized in 34% (86/252) and inappropriately in 2% (3/167) of patients. Despite this new expedited care pathway, some of our ED staff continued to use "traditional" critical care consultation (53%) rather than SSRT activation. This represents a common barrier in healthcare change management, where providers continue to use the more familiar pathways. Whether SSRT underuse is due to under-recognition, disagreement with its use for specific patients, organizational barriers, or a combination thereof, is not clear and would require further investigation. Possible explanations for underuse may be barriers related to physician knowledge or attitudes regarding the SSRT (25). For example, it is possible that physicians failed to recognize that patients were eligible for SSRT activation (e.g., hypoperfusion signs, occult shock). We also found that median APACHE IV score was higher in the group receiving ICU consultation compared to the group receiving SSRT activation (Appendix 1). Therefore, physicians may have decided to implement ICU consultation in more severely ill patients in anticipation of the need for ICU admission. They may have chosen not to use the SSRT in others based on objective data or inherent biases, which we did not set out to measure in our study.

The outcomes of our study suggested that computer mediated screening tools are valuable to identify sepsis patients; however cannot replace clinician's assessment to diagnose severe sepsis. Easily identified, clear-cut criteria for sepsis and septic shock as defined by *Sepsis-3* are easy to identify with an HER (36). The more subtle criteria that constitute severe sepsis may be missed without astute human assessment and decision to intervene. By using the CSSA we increased the identification of septic patients; with the addition of adding the variable of "human decision making" on whether or not to activate the SSRT, we observed sepsis related hospital mortality rate (9.4%) which was lower than the reported United States mortality rate for septic patients (1, 37). We also observed that our septic shock mortality rates 13.5% (34 out of 252) are even lower than those reported in ProCESS, ProMISe and ARISE trials (38-40). A fundamental difference is that instead of relying on the human factor recognition of sepsis by a provider or research coordinator in the ED we are utilizing an automated computerized algorithm that helps with earlier identification. The whole premise of earlier resuscitation improving outcomes implies that by identifying earlier and using a dedicated sepsis and shock resuscitation team that might explain why the outcomes achieved are better than expected.

Evaluation of the specific reasons for underutilization of the SSRT would require further study, however, to overcome barriers to SSRT activation we provided educational materials including a provider pocket cards and a SSRT activation flow sheet (Online supplemental material). Additionally, we also implemented simulation training for ED nurses related to sepsis identification, care algorithms, standardized treatment protocols, and clinician roles and responsibilities. This QI supported the results of other studies incorporating a standardized process via education, interdisciplinary patient management, and visual tools, resulting in clinically significant results (14, 15, 41). Although numerous successful reports on the multidisciplinary approach have been published, limited publications on the human decision to activate sepsis response teams were found.

Limitations

There are various important limitations to our study. First of all, we recognize that

pertinent information regarding the complex human algorithm preceding the decision to utilize the SSRT may not have been fully evaluated. Additionally, a cause-and-effect correlation could not be established through our observational research design. Moreover, we did not abstract information on additional essential elements of treatment (e.g., early and appropriate antibiotics) that have an effect on the clinical outcomes of septic patients. An important limitation is that we did not capture what other algorithm management may have been followed if only ICU consultation was used without SSRT activation. We could not ascertain how outcomes were influenced by underuse of the SSRT given confounding variables. Another limitation is the generalizability of our study and applicability of its results. Nevertheless, some of the challenges that we observed are likely to be experienced at other institutions as well. Our sample size was small and therefore firm conclusions about mortality reduction cannot be drawn from this study. Finally, we identified all patients with sepsis using administrative claims data. If we missed any sepsis patients by using this method, this number would likely be very small.

The well-publicized document, *Sepsis-3,* in February 2016 incorporated the Sequential Organ Dysfunction Assessment (SOFA) score into the diagnosis of sepsis (36). Emergency department providers were resistant to using the SOFA criteria, as it was developed to predict mortality from sepsis, not to diagnose sepsis. The ICU and ED had previously used the same visual algorithm for the SSRT, however, the SOFA criteria was not applicable to the ED setting. Additionally, *Sepsis-3* re-defined the terms "sepsis" and "septic shock," while eliminating the term "severe sepsis." "Severe sepsis" was eliminated citing that sepsis by definition is lethal, thus using the term "severe" related to sepsis is repetitive (36). Although the term "severe sepsis" was removed from the spectrum of sepsis, the project team encouraged the term to continue to be utilized in the updated SSRT visual algorithm. This concurrent event may have been a source of persistent confusion with the new algorithm utilizing the retired term.

Conclusions

Our two-step machine-human interface approach to patients with sepsis utilized an outstandingly sensitive and specific electronic tool followed by more specific human decision-making. As a frequent barrier to early diagnosis and treatment of the septic patient, this approach showed a good balance of sensitivity and specificity, which could allow both better triage and more timely management for different levels of severity of illness while maintaining good outcomes. As with any QI effort, continued education for ED providers would benefit the overall utility of the SSRT resource resulting in improved bundle compliance and patient outcomes. Further research should evaluate provider's reasons for the critical decision of activating versus not activating the SSRT resource.

What is already known on this topic

Early alerts and prompt management of patient with severe sepsis and septic shock (SS/S) starting in the ED have been shown to improve mortality and other pertinent outcomes. Current published evidence reports a wide variety of cut-off criteria for electronic alerts using data from EHR, and several algorithms for treatment that have been applied in different clinical settings. These published studies endorse a more timely response but fail to consistently report a significant improvement in patient outcomes when applied in non-ICU settings.

What this study adds

Our study highlights the value of early recognition by a computer algorithm to identify sepsis; the key element is that computer algorithms still lack the ability to correctly diagnose severe sepsis. We improved timely identification of septic patients by using the CSSA. The addition of "human decision making" input, whether or not to activate the SSRT, coincided with the prospective decrease in sepsis related hospital mortality rate,

which could have been also influenced by other factors, not necessarily related solely to our study activities. We suggest a sequential CSSA followed by the human factor in the form of an experience clinician to assess the patient for severe sepsis yields the best outcomes.

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Lung Cancer: Preventable Disease

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Objective of the paper is to present lung cancer as preventable disease based on epidemiological, molecular and genomic data. Lung cancer is the most deadly malignancy around the world, both in male and female population. Vast majority of lung cancers (close to 90%) are directly caused by cigarette smoking, and thus present one of the most preventable deadly disease in humanity. Analysis of history of cigarette consumption and rise of lung cancer as world epidemics. Review of efforts to fight tobacco epidemics and how it influences incidence and prevalence of the lung cancer. Investigation of the effects of cigarette smoking on health and economic status of Bosnia and Hercegovina. Tobacco epidemics and lung cancer can be prevented. Goal is to exterminate cigarette smoking. That can be achieved only concerted effort by members of family, patients themselves, physicians, researchers, non-governmental organizations, political figures and society as a whole. **Conclusion.** In country like Bosnia and Herzegovina first step is to inform society about devastating effects of cigarette smoking. Best practices already exist and initial goal should be to start using them.

Introduction

Tobacco use presents the largest preventable cause of death and disability in developed and more and more in developing countries. It caused 100 million deaths in 20th century and if current trend continues it will cause up to 1 billion deaths in 21st century. It is responsible for 30% of all cancer deaths and 80% of lung cancer deaths and is associated with increased risk for at least 17 types of cancer (1). Very clear scientific evidence of causality between tobacco smoking and lung cancer was present since 1950's pioneering work by Wynder and Graham (2) as well as Doll and Hill (3). However it took 10 years for information to penetrate into public domain after 1964 Surgeon General's Report in which cancers of the lung and larynx in men (not women) were casually linked to cigarette smoking (4). The topic of smoking was then again addressed in the 1990 Surgeon General's Report on smoking cessation (1990) and in 1982 report which focused on cancer. In 2001 first report on women and smoking was published (USDHHS 2001) (4). Ultimately, report by Office of the Surgeon General (US): Office on Smoking and Health (US) published in 2004 clearly informed general public about lethal risks of cigarettes smoking and particularly emphasized advantage of smoking cessation based on Surveillance, Epidemiology and End Results (SEERS) data showing decline in rates of cancer from 1991 which coincided with decline in smoking among men and less by women (4). However, despite of all these efforts, lung cancer is still leading cause of cancer related deaths both in men and women. Estimated number of deaths from lung

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cancer in USA for 2017 is 155,870 (84,590 males and 71,280 females) (5). In addition to terrible human toll and emotional suffering of hundreds of thousands of families, economic burden on society is enormous. From 2000-2002 the United States spent approximately $ 193 billion each year on tobacco related illnesses and lost productivity because of tobacco-related premature deaths (1). Loss in productivity and increased health care costs associated with second-hand smoking were reported in 2005 to have cost the United States an additional $ 10 billion per year (1).

That picture looks grimmer in country like Bosnia and Hercegovina (BIH). According to Tobacco Atlas more than 8600 deaths per year are attributed to tobacco-related illnesses (6). Still, more than 2000 children (age 10-14) and 1053000 adults (15+ years old) continue to use tobacco each day. That means that 47.2% of men and 30% of women in BIH smoke cigarettes. That number is higher than on average in high-Human Development Index (HDI) countries. The economic cost of smoking in BiH amounts to 891 million KM. that includes direct cost related to healthcare expenditures and indirect costs related to loss of productivity due to early mortality and morbidity. That represents great burden on country that is still recuperating from devastating war and enormous loss of lives, most of them in full productivity age. Interesting economic facts is that tobacco growing is only small fraction of agriculture in BIH with only 0.07% of agricultural land devoted to tobacco cultivation (6). Cigarette imports exceeded cigarette exports in 2016, contributing to country's trade disbalance. It is important to register that more than 80% of 8 million tobacco-related deaths by year 2030 will be in low- and middle-income countries. Since BIH is part of that world, fight against cigarette smoking has to become main focus of activity of multiple levels of society.

Purpose of the paper is to present lung cancer as preventable disease through reduction in cigarette use.

What to do?

In 2003 American Society of Clinical Oncology (ASCO) released policy statement on tobacco cessation and control, which set forth specific recommendations and called for personal accountability in eradicating tobacco use domestically and globally (7). In 2013 Update (1) sets forth a refined set of recommendations for addressing tobacco cessation and control based on updated challenges and opportunities. Recommendations include: 1. Education and awareness, 2. Access to proven Tobacco Cessation Interventions, 3. Tobacco Cessation as a Component of High-Quality Cancer Care, 4, Research on Tobacco Use and Cessation, 5. US Tobacco Regulations, 6. Global Tobacco Control, 7. Leading by Example As Oncology Professionals. This Update very strongly emphasized role of physicians, particularly Oncology providers in tobacco cessation and control. Paper recommended incorporation of five A's (Ask, Asses, Assist, Advise, Arrange) of tobacco cessation in Oncology practice. Most important recommendation, in my opinion, is to ASK every patient if they are using tobacco. Without that question, other A's cannot be used. Unfortunately, in many practices in US and around the word that question is never asked. There are many reasons for that, particularly in countries where tobacco use is part of local culture and where many physicians are tobacco smokers. That raises important question of physician's education about devastating health effects of cigarette smoking. In my opinion, that is of utmost importance, particularly in countries like BIH. Oncologists have important role, since they are physicians that face and treat the most devastating effects of tobacco smoking and also deal with patients

who are greatly motivated to stop using tobacco. Physician-relayed advice on smoking cessation increases the likelihood that patients will try to quit and enhances the odds that those who do so will remain tobacco free (1). Long-term cessation rates include 15% with counselling, 22% with medication alone and 22% to 28% when counselling in combined with pharmacotherapy (8).

Tobacco regulations are becoming very powerful tool in the curbing tobacco epidemic. In 2007 the Institute of Medicine issued a blueprint for US for ending tobacco epidemic (9). It emphasized several tobacco controlling strategies, including financial support of comprehensive state tobacco control programs at the Center for Disease Control and Prevention (CDC)-recommended levels (support for quit lines), increased tobacco taxes as a means to discourage tobacco use and stronger federal regulations and oversight of tobacco products (10). In 2009, the family Smoking Prevention and Tobacco Control Act (FSPTCA) became law, granting the Food and Drug Administration (FDA) authority to regulate the manufacture, distribution and MARKETING of tobacco products to protect public health. The FDA is tasked with aggressively restricting youth access, assessing tobacco industry research on the health and addictiveness of their products, reviewing product ingredients and additives, providing marketing orders and reviewing any health claims made by tobacco companies (11). In 2009, the US Congress voted to increase the federal tax on cigarettes via the Children's Health Insurance Program Reauthorization (12). There is substantial evidence showing that increases in the prices of tobacco products help discourage the use of such products, especially for young children, teenagers and low socioeconomic groups (13). On a global level WHO Framework Convention on Tobacco Control (FCTC) health treaty came into force in 2005 with ratification by the first 40 countries. At the present there 168 countries are signatories and 181 are parties to the WHO FCTC (14). This is comprehensive treaty, which, if effectively enforced in each country, will be deterrent to the globally growing tobacco epidemic, particularly in growing economies. In October 2011 the European Commission committed 5.2 million euros to the FCTC Secretariat to assist low- and middle-income countries to implement convention (15). However, obviously amount of money invested in this Treaty is dwarfed by marketing budget of tobacco companies. The combined revenues of the world's six largest tobacco companies in 2016 were more than $ 364 billion (6). It is 1944% larger than the Gross National Income of BIH. That illustrates uphill battle against tobacco epidemics, particularly in small economies. BIH is not signatory state, but in 2009 became party to the Treaty (Accession). Despite of financial handicap, participation in the Treaty opens possibility to overcome political lobbying and commercial forces supporting tobacco use.

Which of the present best practice measures to reduce tobacco epidemics are presently used in BIH? Very few:

1. Protect from smoke. Best practice is for all public places to be completely smoke-free. At the present NONE of the public places, including health care facilities, educational facilities, government facilities, restaurants, indoor offices, public transportations, and bars are smoke-free (6);
2. Raise taxes on tobacco products. At the present BIH is very close to WHO Benchmark (minimum 70% of retail price to be excise tax). In BIH tax is 68.67% (6). Still more can be done;
3. Offer help for tobacco cessation. Very few. There is no National Quit line. However, considering political landscape, it is more realistic to work on getting Quit lines at the Cantonal level or level of Federation and Republika Srpska;

4. Warning labels. There is no information on percentage of packages with warning labels and there is no warning labels on plain packaging (6);
5. There is no reliable information on anti-tobacco campaigns, although these campaigns were proven to be very effective tool for smoking reduction. This is area where most effort should be concentrated and with involvement of physicians, educators, public, media, public relations and non-governmental organizations (NGO) results could be very significant;
6. Enforce ban on advertising. It seems there is lot to do in enforcing direct ban (TV, radio, magazines, newspapers, internet, billboards) and indirect (appearance of tobacco brands in TV and movies). Camel Joe cartoon character was banned in USA in 1997 as a part of $ 368.5 billion settlement with tobacco producer RJ Reynold. This character was considered as attempt to target younger audience and cultivate new generation of tobacco users. It is banned from public places, TV, billboards. Same happened to Marlboro Man in 1999. It is time to retire or ban all advertising for tobacco everywhere.

At the present it is very clear that vast majority of lung cancers are directly caused by smoking. Figure 1 clearly shows almost identical shapes and parallel distribution of curves representing per capita cigarette consumption and male lung cancer death rate in USA. Lung cancer death curve lags cigarette consumption curve by 20-25 years, illustrating late effect of smoking on lung cancer formation. This confirms quoted data from SEERS showing decline in rates of cancer with decline in cigarette smoking (4). Couple of questions is always raised in discussion about causality between cigarette smoking and lung cancer.

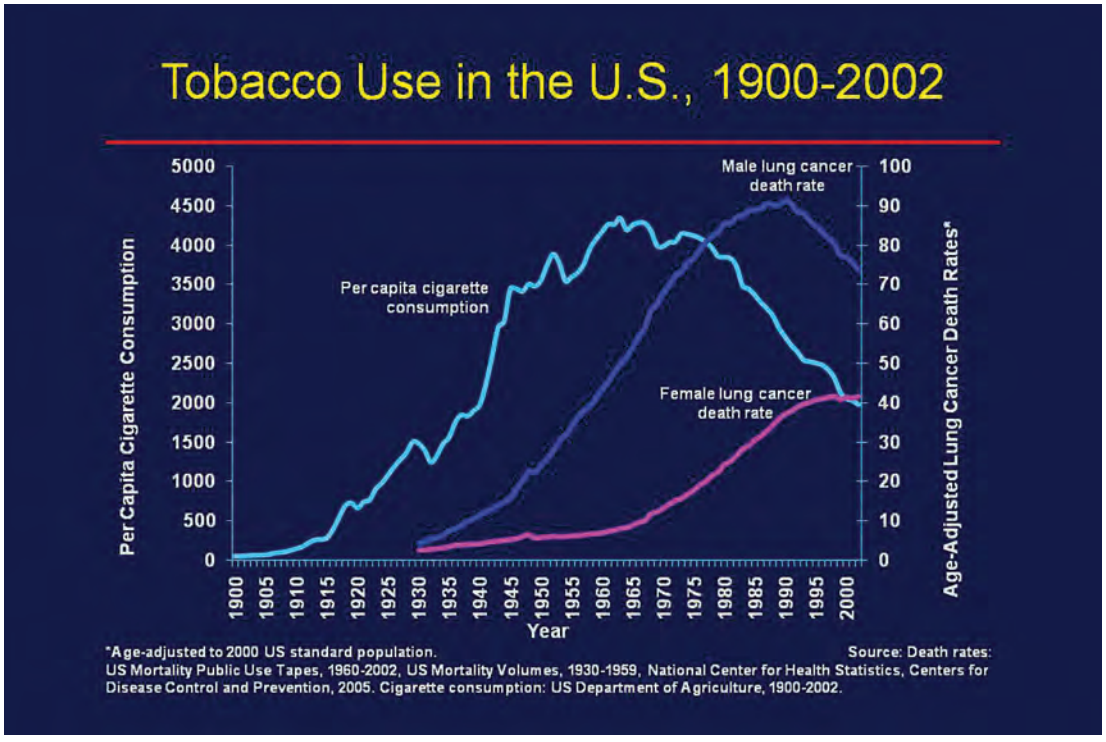

Figure 1. Tobacco use in the United States 1900-2002.

First question is: Why non-smokers get lung cancer?

During smoking of cigarettes, cigars, pipes and other tobacco products, in addition to the mainstream smoke drawn and inhaled by smokers, a stream of smoke is released between puffs into the air from the burning cone (16). Secondhand tobacco smoke is composed of aged exhaled mainstream smoke and diluted side stream smoke. The absolute and relative quantities of many of the individual constituents of side stream smoke are different from those found in mainstream smoke. Exposure to second hand smoke can take place in any of the environments where people spend time. Some studies suggest that exposure to second hand smoke is related to occupation and socioeconomic status, and that higher exposure is more common among adults employed in blue-collar jobs, service occupations and poorly paid jobs and among less well educated (16). Exposure to second hand tobacco smoke may also be higher among racial and ethnic minorities in areas in USA, although it is unclear if this is due to different socioeconomic status (17). Because the home is predominant location for smoking, children are exposed to tobacco smoke as they go about their daily lives. The exposure at home may be added to exposure at school and in vehicles. Consequently, in many countries, children simply cannot avoid inhaling tobacco smoke (18). What are proofs that second hand smoking is associated with lung cancer? It is based on number of case-control and cohort studies. The most commonly used measure of exposure to second hand smoke has been from spouse. This is well defined and has been validated using cotinine studies of never smokers who do or do not live with smokers (16). Other measures of exposure are not so well validated. There have been eight cohort studies of nonsmokers who were followed for years to determine the risk of lung cancer. Six of these studies reported the risk of lung cancer associated with exposure to second hand smoke from spouse (16). All six studies found that the risk for nonsmoking women with partners who smoked was higher than those whose partners did not smoke. In both cohorts that reported on the effect in nonsmoking men whose wives smoked, the relative risk was increased. Many case control studies have been undertaken in several countries (mostly China and the USA). In these studies lung cancer cases were ascertained and matched with controls (usually for age and other factors). Takin the crude relative risks or the adjusted estimates when the crude ones are no available, 25 of the 40 case control studies of non-smoking women showed an increased risk (16). In total 23 studies have been published on exposure to second hand smoking at the work place. Only one study reported a statistically significant association between exposure to second hand smoke at the workplace and risk of lung cancer (19). Since all of these studies were cohorts or case-control studies, based on relatively small number of lung cancer cases and did not have enough power to generate statistical significance on their own, meta-analyses were performed with the aim of pooling the available data and providing more precise estimates of risks. Updated meta-analyses showed that among nonsmoking women who lived with a spouse who smoked, the risk of lung cancer was increased by 24% (relative risk, 1.24; 95% CI, 1.14-1.3) (16). Regarding exposure at the workplace the increased risk for lung cancer in nonsmoking women is about 20% (relatives risk 1.19; 95% CI, 1.09-1.30). Meta-analysis identified statistically significant increase in risk among women exposed to send-hand smoke from the mother during the childhood (50% increases in risk, but wide CI 4-114%). There was lower and nonsignificant increase in risk for exposure to second hand smoke from the father (25%)

(16). Beside second hand smoking there are other occupational and environmental factors that can contribute to rise of lung cancer in relatively small, but real number of non-smokers, not exposed to second hand smoke. List is large, and we will mention only established carcinogens (inorganic arsenic, asbestos, ether, chromium compounds, gamma radiation, X-rays, mustard gas, nickel compounds, polycyclic aromatic hydrocarbons, and radon decay products, soots, tars, mineral oils, vinyl chloride, and wood dust).

Another burning question: Why only small percentages of smokers get lung cancer?

Lung cancer is complex disease and multiple factors contribute to its raise and progression. One of the major factors is host susceptibility (20). Epidemiologic studies showing an association between family history and an increased risk of lung cancer provided the first evidence of host susceptibility. Susceptibility and risk are clearly increased in inherited cancer syndromes caused by rare germ-line mutations in p53, retinoblastoma

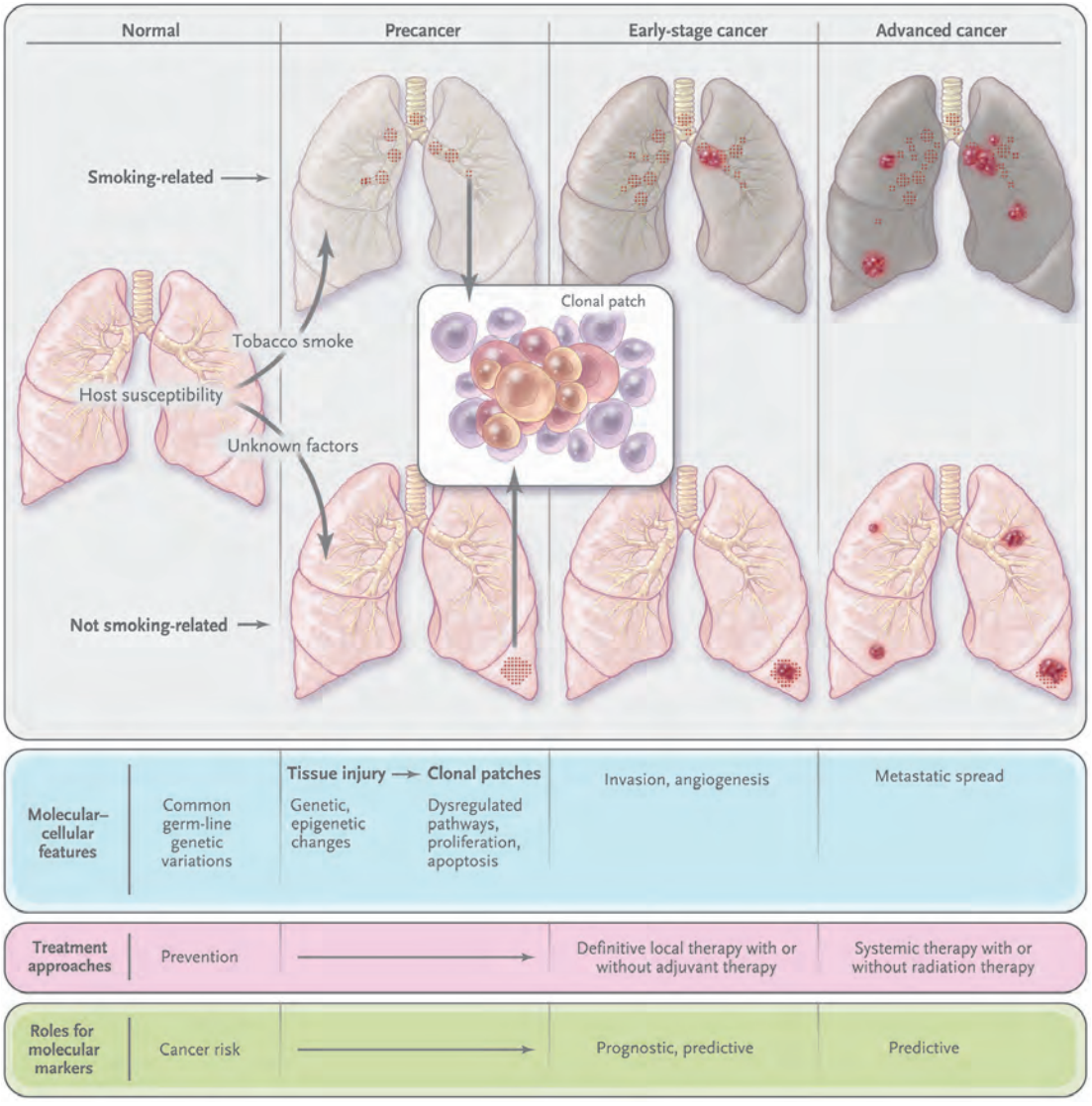

Figure 2. Molecular Evolution of Lung Cancer [Reprinted with Permission from N Engl J Med.] (20).

Table 1. Genetic Abnormalities Specific in the Lung to Non–Small-Cell Lung Cancer and Small-Cell Lung Cancer.*

Abnormality	Non–Small-Cell Lung Cancer		Small-Cell Lung Cancer
	Squamous-Cell Carcinoma	Adenocarcinoma	
Precursor			
Lesion	Known (dysplasia)	Probable (atypical adenomatous hyperplasia)	Possible (neuroendocrine field)†
Genetic change	*p53* mutation	*KRAS* mutation (atypical adenomatous hyperplasia in smokers), *EGFR* kinase domain mutation (in nonsmokers)	Overexpression of c-MET
Cancer			
KRAS mutation	Very rare	10 to 30%‡	Very rare
BRAF mutation	3%	2%	Very rare
EGFR			
Kinase domain mutation	Very rare	10 to 40%‡	Very rare
Amplification§	30%	15%	Very rare
Variant III mutation	5%¶	Very rare	Very rare
HER2			
Kinase domain mutation	Very rare	4%	Very rare
Amplification	2%	6%	Not known
ALK fusion‖	Very rare	7%	Not known
MET			
Mutation	12%	14%	13%
Amplification	21%	20%	Not known
TITF-1 amplification	15%	15%	Very rare
p53 mutation	60 to 70%	50 to 70%‡	75%
LKB1 mutation	19%	34%	Very rare
PIK3CA			
Mutation	2%	2%	Very rare
Amplification	33%	6%	4%

* Non–small-cell lung cancer includes squamous-cell carcinoma and adenocarcinoma.
† Neuroendocrine fields have been detected only in tissue surrounding tumors and have been characterized by extremely high rates of allelic loss and by c-MET overexpression (Salgia R: personal communication).
‡ Variations are based in part on smoking profiles.
§ The percentages include increased gene copy number from amplification or polysomy and represent percentages from resected cancers. The percentages are higher in primary tumors from patients in whom metastatic disease develops. Increased copy numbers have been reported in squamous dysplastic lesions but not in adenocarcinoma precursors.
¶ Genomic *EGFR* variant III mutations have been detected only in lung squamous-cell carcinoma, and these tumors are sensitive to irreversible EGFR tyrosine kinase inhibitors. The incidence of 5% is substantially lower than that of 30 to 40% for the detection in squamous-cell carcinoma or adenocarcinoma by immunohistochemical analysis or other techniques.
‖ The anaplastic lymphoma kinase (*ALK*) fusion gene (involving chromosome 2p), consisting of parts of *EML4* and *ALK*, is transforming in fibroblasts and occurs in adenocarcinoma but not in other types of non–small-cell lung cancer or other nonlung cancers.

Table 1=Reprinted from permission from N Engl J Med. (20).

and other genes, as well as germ-line mutations in the epidermal growth factors receptor (EGFR) gene (21-24). More recently, three large genome wide association studies identified an association between single-nucleotide polymorphism (SNP) variation at 15q24-15q25.1 and susceptibility to lung cancer (20). The region of the SNP variation was linked to lung carcinogenesis and includes 2 genes encoding subunits of the nicotinic acetylcholine receptor alpha, which is regulated by nicotine exposure (25, 26). Lung cancer susceptibility and risk also increase with reduced DNA repair capacity (particularly when accompanied by exposure to tobacco smoke) that results from germ-line alterations in nucleotide excision repair genes, such as ERCC1 (27, 28). An-

other contributing factor to the rise of lung cancer is clonal evolution. Changes in certain genes occur in both nonmalignant lung tissue of smokers and patients with lung cancer pointing to diffuse lung injury (20). This is consistent with theory of "field carcinogenesis" or "field cancerization" (29). Molecular evolution of the lung cancer is presented in Figure 2.

Early events in the development of non-small-cell lung cancer include loss of heterozygosity (LOH) at chromosomal region 3p21.3, 3p14.2, 9p21 (p16) and 17p13 (p53). All these genes are tumor-suppressor genes. LOH patterns in squamous cell and adenocarcinoma differ (chromosome 3p deletion is much more extensive in squamous cell carcinoma) (20). Mutations in the EGFR kinase domain occur early in the development of adenocarcinoma that is generally unrelated to smoking, and KRAS mutations occur early in the development of smoking related adenocarcinoma (30, 31). Genetic abnormalities specific in lung cancers are presented in Table 1. In addition to these abnormalities, chromosomal rearrangement of the gene encoding ROS1 protooncogene receptor kinase was found to define a distinct molecular subgroup of non small cell lung cancers (32).

What influence genetic abnormalities related to lung cancer have on oncology practice?

There is a sea of change in how we approach lung cancer in every day's practice. Recently, treatment paradigms for non-small cell lung (NSCLC) which account for 80-85% of all lung cancers have shifted from one based on histology (adeno-, squamous- and large-cell carcinoma) to one that incorporates molecular subtypes involving particular genetic alterations that drive and maintain tumorigenesis (33). Story started with identification of somatic mutations in the tyrosine kinase domain of the EGFR gene in 8/9 patients who responded to tyrosine kinase inhibitor (TKI) gefitinib, while none of the 7 patient who did not have mutation showed response (34). Subsequently it was found that mutations in the EGFR most commonly deletions in exon 19 affecting the amino acid motif LREA (delE476-750) or substitution of arginine for leucine at position 858(L858R) in exon 21 are present in approximately 17% of tumors in patients with pulmonary adenocarcinoma and lead to constitutive activation of EGFR tyrosine kinase (35). The mutations occur with increased frequency in women and nonsmokers. Responses to EGFR TKI's in patient carrying these activating mutations were much higher (78%) than what has been described in patients treated with standard platinum –doublet chemotherapy (25-30%). The vast majority of patients who have an initial response to EGFR TKI's will eventually relapse. Recent studies identified EGFR T790M mutations (in exon 20) as a main culprit for lack of response or relapse in patients with EGFR gene mutations after treatment with standard EGFR TKI's . The binding kinetics of the mutant EGFR appears to be altered by the T790M mutation (20). New irreversible EGFR inhibitors suppress T790M-mutatnt tumor cells are showing now to be effective treatment for patients carrying that specific mutation. Other activating mutation that has major effect on biology and lung cancer progression is KRAS mutation which limited to NSCLC, predominantly adenocarcinoma and is virtually mutually exclusive of mutations in EGFR kinase domains and is associated with resistance to EGFR TKI's and chemotherapy. Most KRAS mutations are smoking-related G to T transversions and affect exon12 (90% of patients) and exon 13 (36). A distinct KRAS mutational profile consisting of G to A transition mutation was recently detected in non-smokers and is on unclear functional significance (20). Transversions

(smokers) and transitions (non-smokers) also have been reported for p53 mutations in lung adenocarcinomas (36). Activating mutations or translocations of the anaplastic lymphoma kinase gene (ALK) have been identified in NSCLC by Japanese group (37). In NSCLC EML4-ALK is an aberrant fusion gene that encodes a cytoplasmatic chimeric protein with constitutive kinase activity (36). Multiple distinct EML4-ALK chimeric variants have been identified, representing breakpoints within various EML4 exons. This genetic mutation is not common occurring in 2-7% of all NSCLC and is more prevalent in patients who never smoked or who have history of light (short period and small number of cigarettes) smoking (38). However considering very high incidence and prevalence of lung cancer, we can estimate that 7,800 patients in USA and 60,000 worldwide will every year be diagnosed with EML4-ALK positive NSCLC. That particularly becomes important in the light of very effective treatments with first, second and now third line selective ALK inhibitors. It is interesting that first generation ALK inhibitor crizotinib showed great efficacy in patients with ROS1-rearranged NSCLCA (39). The ROS1 oncogene encodes an orphan receptor tyrosine kinase related to ALK, along the members of the insulin receptors family. ROS1 is activated by chromosomal rearrangement in variety of human cancer including NSCLC. Rearrangement leads to fusion of a portion of ROS1that includes the entire tyrosine kinase domain with 1 of 12 different partner proteins (39). The resulting ROS1 fusion kinases are constitutively activated and drive cellular transformation and proliferation. ROS1 rearrangement occur in approximately 1% of patients with NSCLC and approximately 1,300 in US and 15,000 worldwide cases will be driven by oncogenic ROS1 fusion. It is again more frequently found in non-smokers and patient with history of light (short period and small number of cigarettes) smoking. Another mutation that could be important in the treatment of NSCLC is mutation in B-Raf proto-oncogene, serine/threonine kinase (BRAF). Mutations in BRAF, observed in 2%–4% of NSCLCs, typically lead to constitutive activation of the protein and, as a consequence, lead to activation of the mitogen-activated protein kinase signaling pathway. Direct inhibition of mutant BRAF and/or the downstream mitogen-activated protein kinase (MEK) has led to prolonged survival in patients with *BRAF*-mutant metastatic melanoma (40). Same treatment is showing promise in the treatment of patients with NSCLC.

Conclusion

In conclusion lung cancer is very heterogeneous disease with high tumor mutational burden and very hard to treat when metastatic. However, it is preventable disease in very high percentage of cases (85-90%). Goal is to exterminate cigarette smoking. That can be achieved only concerted effort by members of family, patients themselves, physicians, researchers, non-governmental organizations, political figures and society as whole. Best practices already exist and initial goal should be to start using them. BIH society needs to make steps toward adopting all these strategies. Otherwise, cost in human life will continue to grow.

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Epidemiological, Clinical and Molecular Characterization of Human Brucellosis in Bosnia and Herzegovina – An Ongoing Brucellosis Outbreak

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Objective. The aim of this study was to evaluate an ongoing outbreak of brucellosis in southern region of Bosnia and Herzegovina (BIH) on the epidemiological, clinical and molecular level. **Patients and methods.** This study included 19 patients affected by brucellosis between 2015 and 2017, in Trebiševo (BIH). Out of 19 patients, 16 were admitted to and treated at the Department of Infectious diseases of the University Clinical Hospital Mostar, while three patients were treated in ambulatory care setting. Epidemiological, clinical and microbiological parameters were investigated. The Rose Bengal test (RBT) positive sera were serologically confirmed by complement fixation test (CFT). We also analyzed blood cultures, and isolates were additionally serotyped. Molecular analyses were performed with Bruce-ladder multiplex polymerase chain reaction (PCR) and multiple locus variable number of tandem repeat analysis of 16 loci (MLVA-16) assay. **Results**. Fifteen out of 19 patients had been professionally exposed to the bacterium, while four patients acquired brucellosis without prior contact with infected animals. In seven out of eight (87.5%) patients with localized form of brucellosis, we detected significantly higher values of C-reactive protein (CRP) or erythrocyte sedimentation rate (P<0.001). *B. melitensis* was isolated from 13/16 (81.3%) blood culture samples, and additionally serotyped as biovar 3. Using MLVA-16 assay, 11 isolates were genotyped. We observed complete genotype matches among 8/11 *B. melitensis* isolates, while 3/11 isolates differed in Bruce04 locus. **Conclusion**. Overall, our study confirms the usefulness of MLVA-16 method in the epidemiological and molecular research of brucellosis during epidemic that, most likely, originated from the same source.

Introduction

Brucellosis is an emerging zoonotic infection caused by the bacterial genus *Brucella*. The infection is transmitted to humans from infected animals (most commonly from sheep, goats, and pigs) either by the ingestion of infected animal products, direct contact with an infected animal, or inhalation of infectious aerosols (1).

With more than 500,000 new cases per year (2), brucellosis continues to be the most common bacterial zoonosis worldwide and is among the major public health concerns (1). Since the actual incidence of brucello-

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sis is considered to be 26 times higher than the number of reported cases, in addition to health consequences, brucellosis represents a significant economic and financial burden to the developing endemic countries (1-4). Furthermore, four *Brucella* species, namely *Brucella abortus*, *B. melitensis*, *B. suis* and *B. canis*, are classified as category B bioterrorism agents according to the Centers for Disease Control and Prevention (CDC), as they are moderately easy to disseminate, and they result in moderate morbidity and lower mortality rates (5). Clinically, brucellosis often presents with acute symptoms such as fever, night sweats, arthralgia, myalgia, low back pain, and weight loss, as well as weakness, fatigue, malaise, headache, dizziness, depression, and anorexia. However, brucellosis may also progress to a chronic state, especially if not detected early enough due to nonspecific symptoms (1).

In Europe, brucellosis is prevalent in southern and south-eastern countries, and the highest disease incidence was reported for Greece, Former Yugoslav Republic of Macedonia, and Albania among others (2). In the Mediterranean countries and Bosnia and Herzegovina (BIH), *B. melitensis* is the most frequently isolated species of *Brucella*, which predominately affects goats and sheep (2, 6-9). In BIH, only a few isolated cases of brucellosis were reported between 1980 and 2000 (6, 10). For instance, the first case of an outbreak of human brucellosis in BIH was reported in 1985-1986 on the military polygon Manjača, when approximately 50 people acquired the disease after the contact with infected animals imported from the south-eastern Balkan Peninsula (7, 8, 10). However, during the 2000s, the number of people with brucellosis rapidly increased in BIH, peaking at 33.43/100,000 citizens in 2008 (8).

After the 1992-1995 war in BIH, the first cases of human brucellosis were reported in 1999 and 2000 following the return of refugees to the region of Trebinje (East Herzegovina, Republic of Srpska), where the source of infection was donated livestock. In the same period, another outbreak of human brucellosis occurred in Herzegovina-Neretva canton, i.e., 10 cases of human brucellosis were evidenced in Bogodol and Goranci villages (10).

Nevertheless, after the implementation of the program for mass vaccination of animals in 2009, especially of small ruminants such as sheep and goats, the incidence of human brucellosis in BIH rapidly decreased (8). One of the causes of the persistent occurrence of animal and human brucellosis in countries such as Albania, Macedonia and BIH, is poor veterinary and health care due to a lack of systematic approach to this problem by the local politicians and professionals, during the last decade (11). Consequently, brucellosis in BIH shows a tendency to increase and become the most important public health problem in the country (2, 7, 8, 12, 13)

Among the problems in the epidemiological research of brucellosis is the inability to perform accurate molecular subtyping of *Brucella* isolates, due to a high degree of similarity between the strains (14, 15). Tandemly repeated DNA sequences, such as minisatellites (repeat unit size ≥9 base pairs) and microsatellites (repeat unit size up to 8 base pairs) have been used for the molecular distinction of different bacterial species and strains (14, 16-19). In this context, a technique named multiple locus variable number of tandem repeat analysis (MLVA) has proven to be useful in the genotypic distinction of *Brucella* strains, as well as in distinguishing new infections from relapses in patients with brucellosis (14, 15). The MLVA-16 method met the majority of requirements for bacterial genotyping, including simplicity, typing capacity, repeatability, reproducibility, stability and epidemiological applicability (14, 16-18, 20).

In this study, we clinically and epidemiologically characterized an ongoing outbreak of brucellosis in the southern part of BIH with a focus on the suitability of MLVA method in molecular and epidemiological research of human brucellosis.

Patients and methods

Patients

This study included 19 patients affected by epidemic brucellosis between 2015 and 2017, in Trebiševo (BIH). Out of 19 patients, 16 (84.2%) were admitted to and treated at the Department of Infectious diseases of the University Clinical Hospital Mostar, whereas the remaining three patients were treated in ambulatory care setting, without blood culture examination. We analyzed following parameters:

Socio-epidemiological characteristics e.g., gender, age, profession, way of transmission; Clinical characteristics e.g., characteristics of fever, fatigue, malaise, night sweating, loss of appetite, back pain, splenomegaly, lymphadenopathy, cough, antimicrobial consumption prior hospitalization, chronic diseases; Microbiological characteristics e.g., RBT CFT, blood cultures, genotyping; Laboratory findings e.g., C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), leukocytosis, lymphocytosis >40%, Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT), Lactate dehydrogenase (LDH); Local infections (clinical manifestations or complications) of brucellosis e.g., sacroiliitis, arthritis, spondylodiscitis, endocarditis, orchitis and efficiency in the treatment.

Serological analysis

The Rose Bengal test (RBT). The RBT was used as a screening test for *B. abortus*, *B. melitensis*, and *B. suis.* We performed the test according to the Manual of Diagnostic Tests and Vaccines for Terrestrial Animals using Rose Bengal Brucellosis Antigen (IDEXX, Pourquier, Montpellier, France) (21).

Complement fixation test (CFT). The CFT is a serological test used to confirm the presence of complement fixating antibodies in sera of patients with brucellosis. This method was used as a confirmation test for all RBT positive sera as has been explained previously (22).

Blood cultures

We analyzed the blood cultures of 16 patients hospitalized at the Department of Infectious Diseases, University Clinical Hospital Mostar. BacT/Alert SA (aerobic) and SN (anaerobic) blood culture bottles were aseptically inoculated with 5-10 ml of patients' blood and incubated in the BacT/Alert 3D automated sensor-metric system (bioMérieux, Marcy l´Etoile, France). The bottles were incubated under continuous agitation and monitored for five to seven days or until they became positive for bacterial growth, but no longer than 10 days.

Bacterial detection and isolation

Gram staining was performed for positive blood culture bottles. Based on the staining results, the samples positive for bacterial growth were subcultured on *Brucella* agar (MERCK, Darmstadt, Germany) and incubated at 37°C, in microaerophilic conditions (10% CO2) for at least 5 days. The suspected colonies were presumptively identified by Gram staining, as well as urease (MERCK, Darmstadt, Germany) and oxidase (bioMérieux, Marcy l´Etoile, France) tests. The *Brucella* isolates were characterized based on the colony morphology, positive urease and oxidase results, presence of the typical small Gram-negative coccobacilli, and molecular tests (17, 23, 24). *B. melitensis* isolates were

tested for biovars by their ability to cause agglutination of anti-A, -M and -R monospecific sera (Animal & Plant Health Agency, formerly known as the Animal Health and Veterinary Laboratories, United Kingdom).

Molecular analysis

DNA isolation. DNA was isolated from the bacterial isolates using commercially available QIAamp DNA Mini QIAcube Kit and QIAcube system (QIAGEN, Hilden, Germany) according to the manufacturer's instructions. An amount of 2 or 5 µl of the supernatant was used for further analysis.

Species identification. Bruce-ladder, a multiplex PCR assay able to differentiate between most known *Brucella* species, was used as a reference method for the identification of *Brucella* species (24). The PCR reaction mix (volume 20 µL) included: 10 µL of Multiplex PCR Master Mix (Qiagen, Hilden, Germany), 2.5 µL of RNase-Free Water (Qiagen, Hilden, Germany), 0.4 µM of BMEI0998f and BMEI0997r primers (Invitrogen, UK or Macrogen, Netherlands), 0.1 µM of each of the remaining primers, and 2 µL of DNA. The cycling protocol was used as previously described (24).

MLVA genotyping. MLVA-16 genotyping was performed with 16 gene loci as previously described (14, 17). The loci were classified into three panels: Panel 1 (Bruce06, 08, 11, 12, 42, 43, 45, and 55); Panel 2A (Bruce18 and 21); and Panel 2B (Bruce04, 07, 09, 16, 30, and Bruce19 previously included in the Panel 2A). *B. melitensis* 16M was used as the reference strain for the comparison of genotyping results and verification of test quality.

The PCR reaction mixture (20 µL) was used for all MLVA loci, inlcuding: 10 µL of HotStarTaq Master Mix (Qiagen, Hilden, Germany), 6 µL of water (RNase-free water, Qiagen, Hilden, Germany), 0.5 µM of each primer pair specific to the target locus (Invitrogen, Paisley, UK or Macrogen, Amsterdam Zuid-Oost, Netherlands), and 2 µL of template DNA. The cycling program was used as previously described (14, 17). All amplifications were performed on ProFlex PCR System (Applied Biosystems, USA). The PCR products were analyzed using QIAxcel Advanced system (QIAGEN, Hilden, Germany) with DNA size ladder in the range of 100-2500 base pairs. The DNA fragment sizes obtained with MLVA-16 assay were converted to a number of individual repeats (17). The results were presented in the form of 16-digit numerical codes based on the *Brucella* allele assignment table version 3.6 (25). The results of MLVA-16 were compared to the data deposited in the personal collection of strains at the Croatian Veterinary Institute.

Ethics statement

All procedures followed were in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments. As this was a retrospective database analysis, informed consent was not required and any patient identifying information was omitted.

Statistical analysis

We analyzed the results using IBM SPSS Statistics for Windows, Version 23.0 (IBM Corp, Armonk, NY) and Microsoft Excel (365). The results are presented as absolute numbers (n) and percentages (%). Fisher's exact test was used for categorical data analysis, due to small number of subjects, and values of $P<0.05$ were considered statistically significant.

Results

Out of 19 patients with brucellosis, 11 patients (57.9%) were females (mean age 41.9, age range 11-76) and 8 patients (42.1%) were

males (mean age 41.9, age range 8-73). Three out of 19 patients were children, whereas 16 patients were adults. The first evidenced case of this epidemic was a 13-year old girl hospitalized in the summer of 2015, while the last patient was a 62-year old woman, hospitalized in May 2016. The youngest patient was an eight-year-old boy, whereas the oldest patient was a 76-year old woman. Eleven out of 19 patients (57.9%) had acute brucellosis, while 8/19 patients (42.1%) clinically presented with one of the following localized infections presented either by clinical manifestations or complications such as: sacroiliitis, arthritis, spondylodiscitis, endocarditis, and/or orchitis.

The majority of patients 15/19 (78.9%) were directly involved in sheep farming (adults and farmers' children) and had a close contact with sheep during lambing. In addition, 14/19 patients (73.7%) consumed the traditionally prepared sheep cheese and milk without prior thermal processing. Four out of 19 (21%) patients did not have any prior contact with the animals.

A significant number of patients, 16/19 (84.2%), had fever, fatigue, malaise and sweating. Splenomegaly was observed in 7/19 patients (36.8%), loss of appetite in 10/19 patients (52.6%) and back pain in 11/19 patients (57.9%). Only 3/19 patients (15.8%) did not have any clinical symptoms of brucellosis.

Increased levels of erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) were observed in 12/19 (63.2%) and 11/17 (64.7%) examined patients, respectively. Elevated levels of leukocytes were observed only in two out of 19 patients (10.5%), while in 11/19 patients (57.9%) relative lymphocytosis was present. Increased lactate dehydrogenase (LDH) was observed in 14/19 (73.8%) patients and elevated hepatic transaminases were detected in 8/19 (42.1%) patients (Table 1).

Table 1. Socio-epidemiological, clinical and microbiological characteristics of patients with brucellosis

Characteristics	n/total	(%)
Gender		
Male	8/19	(42.1)
Female	11/19	(57.9)
Age		
Children (8-17 years)	3/19	(15.8)
Adults (>18 years)	16/19	(84.2)
Age range (year)		
8-17	3/19	(15.8)
18-44	6/19	(31.6)
45-64	5/19	(26.3)
>65	5/19	(26.3)
Profession		
Pupils (farmers' children)	3/19	(15.8)
Farmers	12/19	(63.2)
Tourists	4/19	(21.0)
Possible transmission		
Aerosol	3/19	(15.8)
Ingestion	14/19	(73.7)
Contact	15/19	(78.9)
No prior contact	4/19	(21.0)
Fever		
No fever	3/19	(15.8)
Fever <15 days	9/19	(47.4)
Fever >15 days	7/19	(36.8)
Symptoms		
Fatigue	16/19	(84.2)
Malaise	16/19	(84.2)
Sweating	16/19	(84.2)
Loss of appetite	10/19	(52.6)
Back pain	11/19	(57.9)
Splenomegaly	7/19	(36.8)
Lymphadenopathy	2/19	(10.5)
Cough	1/19	(5.3)
Antimicrobials prior hospitalization	3/19	(15.8)
Presence of other chronic disease	5/19	(26.3)
Microbiological characteristics		
RBT (Rose Bengal test)	19/19	(100)
Complement fixation test (CFT)	18/19	(94.7)
Blood culture positive	13/16	(81.3)
Genotyping - performed	11/13	(84.6)

Table 1 (continued): Socio-epidemiological, clinical and microbiological characteristics of patients with brucellosis

Characteristics	n/total	(%)
Genotyping - not performed	2/13	(15.4)
Laboratory findings		
CRP not performed	2/19	(10.5)
CRP <5 mg/mL	6/19	(31.6)
CRP 5-49 mg/mL	7/19	(36.8)
CRP 50-99 mg/mL	2/19	(10.5)
CRP > 100 mg/mL	2/19	(10.5)
ESR <25 mm/h	12/19	(63.2)
ESR >25 mm/h	7/19	(36.8)
Leukocytosis	2/19	(10.5)
Lymphocytosis >40%	11/19	(57.9)
AST >30 mU/mL	8/19	(42.1)
ALT >35 mU/mL	8/19	(42.1)
LDH >225 mU/mL	14/19	(73.7)
Clinical manifestations or complications		
No	11/19	(57.9)
Sacroiliitis	2/19	(10.5)
Arthritis	1/19	(5.3)
Spondylodiscitis	3/19	(15.8)
Spondylodiscitis and endocarditis	1/19	(5.3)
Orchitis	1/19	(5.3)
Efficiency in treatment		
Mortality	1/19	(5.3)
Successfully treated	18/19	(94.7)
Relapses (one year follow up)	0/19	(0.0)

CRP=C-reactive protein; ESR=Erythrocyte sedimentation rate; AST=Aspartate aminotransferase; ALT=Alanine aminotransferase; LDH=Lactate dehydrogenase.

The blood cultures were microbiologically positive for bacterial growth in 13/16 (81.3%) patients and further analyzed with Bruce-Ladder PCR method for the final confirmation of *Brucella* species. The assay showed the presence of *B. melitensis* in all blood culture isolates. According to positive agglutination with anti-A and anti -M monospecific sera all isolated strains were typed as *B. melitensis* biovar 3.

In addition, we investigated the relationship between acute inflammatory markers (e.g., CRP and ESR) and the severity of the disease. The levels of CRP above 20 mg/L were observed in 7/17 (41.2%) patients presented with localized form of brucellosis, while only in one out of eight patients with localized form of brucellosis lower CRP was observed (P<0.001). Similarly, in 7/19 (36.8%) patients with localized form of brucellosis ESR above 25 mm/h was observed (P<0.001). On the other hand, in patients without localized form of brucellosis, CRP below 20 mg/L (in 9/17 patients; 52.9%) and normal ESR (in 11/19 patients; 57.9%) were observed (P<0.001) (Table 2).

Finally, we performed a molecular characterization in 11/13 human blood isolates using MLVA-16. As shown in Figure 1, *B. melitensis* was detected in all 11 isolates, indicating that these 11 patients were part of the same brucellosis epidemic. The Bruce04 and Bruce16 from the panel 2B were the only polymorphic loci.

Table 2**.** Relationship between markers of acute inflammation and severity of brucellosis

Inflammatory markers	Localized infection		P*
	Presence (n; %)	Absence (n; %)	
CRP >20 mg/L	7/17 (41.2)	0 (0%)	<0.001
CRP <20 mg/L	1/17 (5.9)	9/17 (57.9)	<0.001
Total**	8/17 (47.1)	9/17 (52.9)	-
ESR >25 mm/h	7/19 (36.8)	0/19 (0%)	<0.001
ESR <25 mm/h	1/19 (5.3)	11/19 (57.9)	<0.001
Total	8/19 (42.1)	11/19 (57.9)	-

*Fisher's exact test; CRP=C-reactive protein; ESR=Erythrocyte sedimentation rate. **In two patients without localized form of brucellosis CRP was not performed.

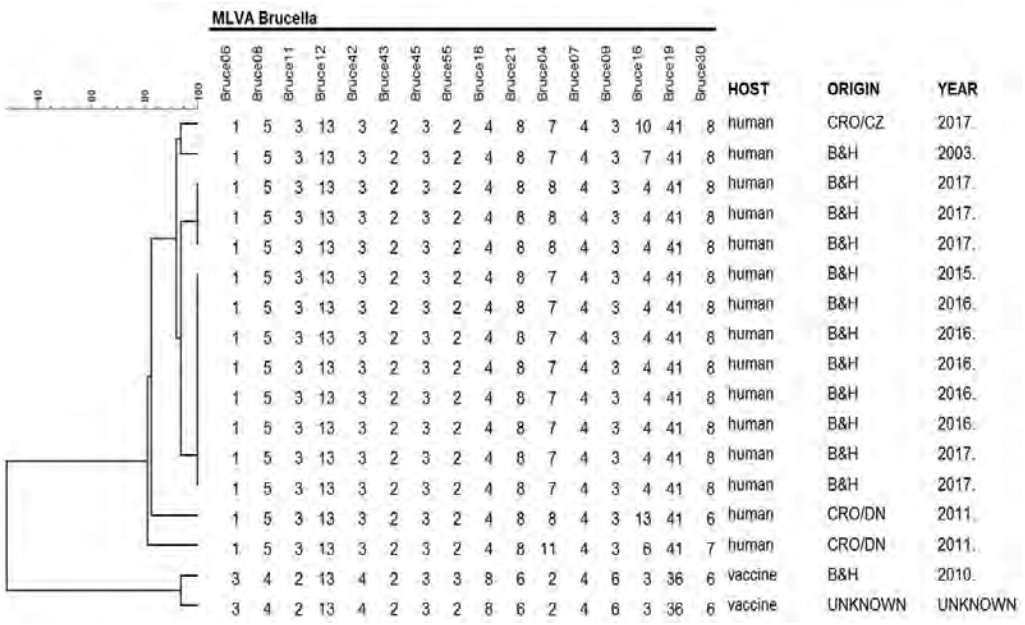

Figure 1. Dendrogram obtained with the results of multi-locus variable-number tandem repeat analysis-16 (MLVA-16) genotyping assay showing the relationships between 11 *Brucella* isolates obtained from patients from Trebiševo (BIH) in the period 2015-2017 and additional five marked strains collected from three individuals from Croatia (Canton Zagreb, CZ; Canton Dubrovnik-Neretva, DN) and one from BIH isolated previously, as well as two *B. melitensis* Rev. 1 vaccine strains. The individual MLVA-16 loci and the number of variable number tandem repeats for each isolate are shown.

During this epidemic, a 65-year old man died from severe complications of brucellosis, i.e., spondylodiscitis and endocarditis resulting in embolization of central nervous system. Eighteen patients (94.7%) were successfully treated without any relapse of the disease in one year follow up.

Discussion

An important point of our study is that this epidemic was related to the specific method of sheep milk collection, followed by the traditional way of preparing sheep cheese in Herzegovina, which does not require prior thermic processing. Another crucial point is that a large number of sheep from different households (more than 15 different farms) were pooled to make a larger livestock group (around 800 animals) on a mountain ranch, where more vegetation is present during the summer time.

This epidemic of brucellosis started in 2015, when we detected two patients who were in direct contact with sheep and had been preparing different sorts of milk products, but without any serious epidemiological and sanitary inspection. Next year, in the autumn of 2016, we registered the second outbreak of human brucellosis when additional 10 patients were hospitalized. Importantly, this epidemic occurred in the same location as in 2015. Since this time more people were affected, a serious veterinary and epidemiological investigation was conducted. Because most of the sheep had not been vaccinated, brucellosis quickly spread among the animals. Moreover, because the vaccination program has not been consistently carried out in BIH (11), during the past years, a significant number of new animals had been acquiring brucellosis and consequently pose a threat to humans. During this epidemic, brucellosis was con-

firmed in 81 out of 800 tested sheep. The infected animals were properly euthanized and disposed outside the inhabited area in Trebiševo, while the rest of the seronegative herd was returned to farms throughout Herzegovina in the late autumn of 2016 (data not shown). Three patients, who had been in a close contact with infected sheep within the farm household, were serologically positive for RBT, whereas most of other family members, who had not had contact with the animals, were negative. In accordance with previous recommendations (26), the seronegative people who had been exposed to the infected animals were continuously tested in the following period, and ten of them were serologically positive for brucellosis, even six months after their last contact with infected sheep. The last outbreak of the disease occurred in the spring of 2017, when we hospitalized and treated seven additional patients with brucellosis. Furthermore, the seronegative sheep were re-tested and most of them were serologically positive for brucellosis, including five cattle in one of the two tested farm households (data not shown). This confirms that *B. melitensis* is highly pathogenic and can cross the species barrier between different mammals (1).

Although brucellosis is a professional disease (6-9, 12, 27-30), here we reported that four out of 19 infected patients had no previous contact with any type of livestock. However, they visited Trebiševo or ate the traditionally prepared cheese in 2016, and most probably acquired the disease either by aerosol or ingestion of the infected food, which has also been reported previously (1, 31-33). Moreover, as ocular infection was suggested by some authors, especially in laboratory conditions, we assumed that some of the patients could have been infected through the conjunctiva (1).

The RBT is still considered to be the best screening tool for brucellosis (34). Three of our patients had no symptoms of brucellosis, and among these, one patient had negative complement fixation test (CFT). On the other hand, RBT was positive in all three patients. This result is in correlation with previously published reports showing a high level of sensitivity for RBT, even as compared with a more specific serological test such as CFT or serum agglutination test (SAT) (34, 35). In the patient with the RBT positive and CFT negative result, *Brucella* was isolated from the blood culture, indicating septicemia. Finally, all isolated strains were typed to belong to *B. melitensis* biovar 3 which is to our knowledge first systematic report of human *B. melitensis* isolates in BIH.

Microbiological isolation remains the "gold standard" for the diagnosis of brucellosis and the sensitivity of this method varies from 15 to 70% (1). Compared to the efficacy of the blood isolation methods in other medical centers in BIH (36), the sensitivity of our method was higher as more than 80% of blood cultures were positive. The high rate of positive blood cultures was the result of our careful planning during the sampling of patients highly susceptible for brucellosis.

In contrast to previously published results, where the majority of patients with brucellosis from BIH were males (6, 8), in our group, brucellosis was equally distributed among the males and females. Moreover, almost a half of our patients had a clinical manifestations of brucellosis, indicating that either the acute disease was not recognized by the primary health service or the patients did not have symptomatic acute disease. Also, there is a possibility that some of patients did not want to visit the primary health service. This suggests that, in the case of epidemic history in the specific region, the primary health care service should be more aware of the infection.

Patients with brucellosis often have elevated ESR and CRP levels (37). In this study, we observed that the elevated ESR and CRP levels were strongly associated with differ-

ent disease presentations. For example, the acute inflammatory markers were markedly higher in our patients with localized form of brucellosis, indicating their role in diagnostics and therapy planning for the patients.

In our study, all patients were treated for at least eight weeks, with minimum two, combined antimicrobial agents. The adults and children >8 years old were treated with a combination of doxycycline 100 mg p.o. twice a day (BID) and gentamicin (5 mg/kg) once a day for two to three weeks, followed by a combination of doxycycline 100 mg p.o. BID and rifampicin 300 mg p.o. BID for at least five additional weeks, but more often up to 12 weeks, especially when septicemia was detected. In the case of focal disease, such as spondylodiscitis, longer therapy may be more beneficial (38). The described therapy resulted in complete remission of brucellosis, with no relapse history.

Finally, we performed the genotype analysis of the *Brucella* isolates and correlated these results to the region of origin of this ongoing brucellosis outbreak in BIH. Similar to a previous report (39), using molecular (MVLA) and serological techniques (antisera M, A and R) we also showed that *B. melitensis* biovar 3 was the predominant strain in BIH. In most of our *Brucella* isolates similar genotypes were present, and only in three patients, all from the same family, small sequence differences were observed for the Bruce04 locus. A high mutation rate for the Bruce04 and Bruce16 loci was also recently reported by Kattar et al. in 42 *B. melitensis* strains isolated from patients from Lebanon (15), as well as by Cvetnic et. al. in 29 *B. melitensis* animal isolates originating from Croatia and BIH (40). Similarly, the *B. melitensis* isolates reported in this study and in the previous studies from BIH and the neighboring countries mostly differed in the locus Bruce16. All these results suggest that the locus Bruce16 is among the most variable loci in the analyzed panels, indicating its importance in the epidemiological and molecular research of *B. melitensis*. Recently, Gyuranecz et al. demonstrated rapid *in vivo* genetic mutations of several variable number tandem repeats loci in *B. canis* isolates from dogs, over a three-month period of outbreak (41), which also could indicate the possibility of minor mutations in some of the variable loci of *B. melitensis* during epidemic.

Conclusion

Overall, our study confirms the usefulness of MLVA-16 method in the epidemiological and molecular research of brucellosis in BIH and neighboring countries. In addition, the genotypes of *Brucella* strains isolated from 2003 up until now from the samples in BIH show high similarity and indicate the presence of a regional epidemic. Future research should analyze this phenomenon in detail, by investigating all cases of the local outbreaks of brucellosis in BIH.

What is already known on this topic

Brucellosis is an endemic disease in BIH for more than 15 years representing an emergent treat for people in the country and region. Clinical and epidemiological characteristics of brucellosis are well known. So far, in BIH only B. melitensis was isolated from humans with brucellosis, but there are no molecular data about circulating genotypes.

What this study adds

To date, there were no studies investigating correlation between molecular and epidemiological characteristics of Brucella spp. in the region. We clinically and epidemiologically investigated an ongoing outbreak of brucellosis in the southern part of BIH with a focus on the suitability of Brucella spp. genotyping by MLVA-method in epidemiological research of human brucellosis. In this study B. melitensis biovar 3 was detected in all investigated isolates. Furthermore, the Bruce04 and Bruce16 were the most polymorphic loci. Although complete genotype matches were detected in the majority of B. melitensis isolates, only few isolates slightly differed in Bruce04 locus suggesting that the strains in this region, most likely, originated from the same source.

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The Medical School of the Catholic University of Croatia: Principles, Goals, Standards and Organization

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Key words: Medical education ▪ Transformative learning ▪ Social accountability ▪ Patient-centered care ▪ Longitudinal integrated community clerkship.

The aim of the study was to present the concept on which the School of Medicine at the Catholic University of Croatia (CUC) will be established. The new School will alleviate the shortage of physicians in Croatia and introduce an innovative form of medical education focused on principles of patient-centered care and social accountability. At the same time, the students will acquire all relevant competencies and levels of knowledge, skills and attitudes that are required by current evidence in medical education, European standards and guidelines for quality assurance at higher education institutions. The four pillars of the CUC Medical School are: 1) distributed medical education that involves health institutions outside major medical centers, 2) the concept of transformative learning, 3) teaching and practicing evidence-based medicine, and 4) implementation of quality management principles supported by information technology solutions for effective management of learning, research and practice. The overall aim of the CUC School of Medicine is to educate and train physicians capable of using best available medical evidence to deliver economically sustainable healthcare that can improve equity and health outcomes in the communities they serve, particularly those that are currently underserved. **Conclusion.** The proposed programme is introducing an original system of modern medical education that insists on developing humanistic aspects of medicine, patient-centred care and social accountability, while maintaining all competencies and knowledge levels that a physician should have according to the current understanding of medical education.

Introduction

Healthcare professionals represent the link between medical knowledge and population. They are the public face of a healthcare system and their education is essential for a well-functioning healthcare system (1). Healthcare systems worldwide are becoming more and more expensive and complex, and healthcare professionals are faced with ever increasing challenges, from the new risks of emerging infectious diseases to environmental pollution and risk behavior, all amplified by mass migrations and epidemiological changes in times when there are still large inequalities between and within countries and regions (1, 2).

Medical education lags behind these newly emerging health needs, primarily due to the fragmented, obsolete, and static

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medical curricula, which create inadequately educated physicians who have to deal in practice with problems quite different from those they learned about from the textbooks (3-5). Their competencies do not necessarily match patient and community health needs; they are not trained to work in teams; they do not consider potentials of specific technology in the context of current medical needs and economic possibilities; they do not provide systematic continuous care to their patients; they are oriented toward hospitals at the expense of family medicine; and they are unprepared to work on improving the healthcare system (1).

Awareness of the fact that healthcare professionals receive inadequate education has led to a range of changes, even revolutions, in medical education, but these efforts have not managed to overcome the weaknesses and inequalities of healthcare systems. The first revolution in medical education occurred at the beginning of the 20th century when the medical curriculum started to rely on science (6). The introduction of clinical problem-based education in the mid-20th century marked the second revolution (7). Today, the third revolution is under way, based on systematic improvement of efficacy of healthcare systems through the adjustment of important professional competencies to specific contexts while relying on global knowledge (1). The gap between the requirements of modern science about medical education and medical curricula is also present in Croatia (8-11). The CUC Medical School may narrow this gap, because it would not be burdened by structural issues of the times passed (5, 12-15).

CUC medical school will advocate a special care for the community, the ill, the weak, and the underserved, ensuring that practical work and skill acquisition take priority. It will apply scientific knowledge resulting from progress in medicine and healthcare to teaching and to the local community, spreading academic atmosphere that will aim to reach even the most distant regions of Croatia. Scientific research will be focused primarily on the issues important for patients, followed by topics important for the community, public, and healthcare professionals (16).

The aim of the study was to present innovative concept of a new medical program that will be based on distributed medical education, transformative learning, teaching and practicing evidence-based medicine, and implementation of quality management principles supported by information technology.

Grounds for launching a new medical school in Croatia

The motive to launch the new medical school in Croatia was in part a response to the current shortage of medical physicians in Croatia (17), as well as the introduction of an innovative and modern system of medical education that fosters humanistic aspects of medicine, patient-centered care, and social accountability, while providing the competences and knowledge that current understanding of the medical education requires from future medical doctors.

The suggested transformation of medical education cannot be carried out through the existing institutions, because the state-funded academic community resists the change and persists in maintaining the present state of healthcare system and income through mechanisms of suboptimal staffing and decision-making (18). In such circumstances, internationally competitive institutions, designed in line with the state-of-the-art standards and not limited by the existing relations, have to be built from the scratch.

The CUC School of Medicine will be a non-profit institution, open to all eligible individuals worldwide. Its main goal will be to create a different profile of physicians,

who will be recognized by their respect for patients and orientation toward non-privileged, underserved and underdeveloped regions. The CUC School of Medicine will strive to increase the sense of social accountability in future physicians according to the Consensus for Social Accountability of Medical Schools (19-21), while providing them with the best scientific and research education. The development of the awareness of the need for community-oriented work and lifelong learning, including postgraduate education, will be an integral part of their medical education. In order to reach these goals, outcome competencies will be clearly defined. The focus of teaching will be on the acquisition and development of competencies through learning by doing, i.e. through practical work with patients and scientific research directed at investigating and promoting the patient-health system relationship, and future development of evidence-based medicine.

The shortage of physicians in Croatia, especially its rural areas, justifies the need for opening a new medical school (17, 22-25). On the list of countries ranked by physician density, Croatia is ranked 34th in Europe, with 259 physicians per 100,000 population. European average is 331 physicians per 100,000 population. Therefore, the number of physicians in Croatia is 22% below the European average. To reach the European average, Croatia needs additional 3280 physicians. If the annual average rate of increase in the number of physicians was 3 physicians per 100,000 population, as it was in 2009, it would take 24 years for Croatia to reach that goal (17, 22).

Physicians are needed in many poor and underdeveloped areas in the world (1), and the strategic option for a medical school would be to sponsor students from such areas and provide them with free education. Furthermore, the CUC School of Medicine would be particularly welcoming to Croats living abroad, by enabling them to study in their old homeland, becoming closer to local people, and earning a globally recognized degree.

Organizational structure: four pillars of the CUC School of Medicine

The four pillars of the CUC School of Medicine (Figure 1) include the following set of principles: a) *distributed medical education*, i.e. carrying out teaching activities outside of major medical centers and bringing academic atmosphere to even the most remote communities, where students would receive most of their practical training, b) *transformative learning*, to help future physicians to actively participate in the transfer of the modern medicine achievements to their communities, c) *teaching and practicing evidence-based medicine*, and d) *quality management* principles supported by information technology solutions for effective management of learning, research and practice, as well as for monitoring/evaluation of the progress.

Patient-centered care and social accountability are the central and most important values on which the CUC School of Medicine is built upon. In recent years, an increasing number of medical schools have accepted the concept that medical education is not only an "assembly line" for the production of new physicians (26). The CUC School of Medicine will incorporate social accountability standards into medical education (27). The Catholic identity of the School will be reflected in cultivating the highest and most noble principles of compassion and care for vulnerable populations and in providing an academic platform where various sciences and professions are explored through perspective of the Christian understanding of the world reality as a whole and humans in particular. The CUC School of Medicine will give highest priority

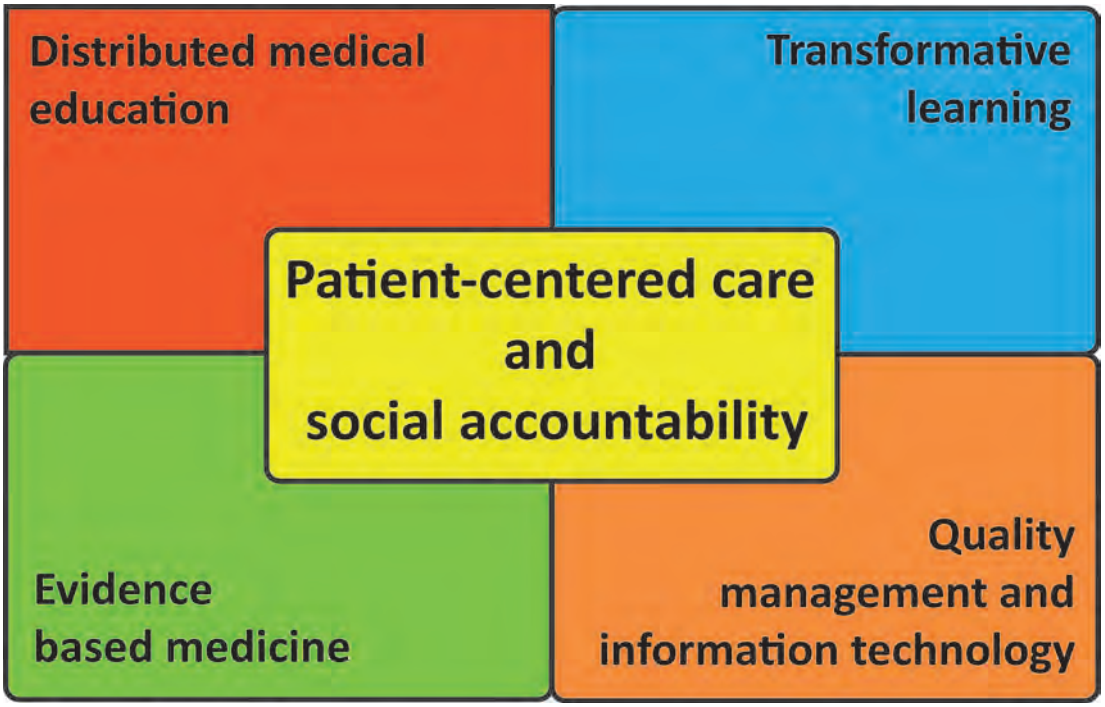

Figure 1. The main determinants of the CUC Medical School organization.

to the development, study, and promotion of the interests of patients and communities and thus, by teaching and setting an example, prevent alienation, commercialization, profiteering, and neglecting the sacredness of life and human dignity in medicine and healthcare. Patient-centered care and social accountability include adequate training in communication skills, medical humanities, and joint patient-physician decision making, while using the best evidence available from the literature in addition to education in social medicine and palliative care (1). Education will rely on particular field-work conditions and involve small hospitals, family medicine practices, and other stakeholders (28).

This focus will be reflected in volunteering and charity work as an integral part of educational process; "verticalization" of medical humanities – i.e. inclusion of medical humanities topics in all years of medical education, which requires the adoption of general principles of treating and communicating with patients; and a large part of the curriculum dedicated to global, social, and palliative medicine. The overall aim is to teach medical students to approach patients with empathy while applying the most recent medical knowledge and technology.

Distributed medical education

The CUC School of Medicine will not have a centralized educational setting. For the first two years, theoretical teaching will mostly be carried out in Zagreb, Croatia, where the CUC is located. Afterwards, students will be distributed to healthcare institutions across Croatia according to the established and carefully planned schedule (29). Thus, students will develop their professional competencies through working with patients in smaller, rural communities, in a specific context of everyday provision of healthcare, and through partnership with local physicians-teachers while using the principles of evidence-based medicine (28).

Not many medical schools in the world provide medical and healthcare education in small provincial hospitals, family medicine practices, and other healthcare institutions in remote areas (28, 30-38). The contact with patients will be based on the concept of continuous *integrated community clerkship* (39-42). The traditional block clerkship, where students acquire clinical experience in a single discipline, has received criticism due to the lack of continuity and inability to follow up the entire process of patient treatment, which leads to the collapse of the concept of patient-centered care (42, 43). Integrated community clerkship is an innovative solution for the limitations of classic clinical clerkship (44, 45).

In CUC concept, a student, who is a clinical clerk, has two mentors – a course mentor, supervising all the students taking his or her course, and a field mentor, taking care of the practical training of individual students. The course mentor communicates with both students and their field mentors using e-technology, evaluates the work of each student and field mentor, keeps records, and administers the exam. The field mentor evaluates practical knowledge of the student, supervises the student's weekly progress in mastering required tasks, and logs reports on student's work into the student's individual portfolio to the course mentor using e-technology.

After a short block of lectures and seminars, the students will be divided into small groups of 2-3 students, and sent to their clinical workplaces outside major medical centers, where they will participate in providing medical care and acquire skills specified by the healthcare program, under the supervision of their field mentor, who they accompany during his or her daily work routine, including on-call shifts. During this practical part of clinical clerkship, each student will have to follow up continually several patients and describe their treatments in the form of writing tasks based on EBM cases (46). The EBM-based writing tasks have to be sent for evaluation and feedback by e-technology to the course mentor, who is usually an experienced and internationally recognized expert. After receiving the course mentor's feedback, each student will present his or her work to the local team. This process includes a three-way exchange of knowledge, where medical procedures carried out by junior mentors/physicians in rural areas are being constantly reviewed.

Selected institutions in small communities will provide clinical training to a limited number of medical students upon signing a collaboration agreement. By signing the agreement, such an institution, which will act as a teaching affiliate, becomes an affiliate institution of the CUC.

In addition to the usual programs of student exchange, e.g. Erasmus exchange program (47), the curriculum involves volunteering in social and healthcare institutions and extensive summer training abroad in both developed and poor regions. The latter will be organized in cooperation with Catholic missions in developing countries.

Providing accommodation to students during their field training is feasible because the number of enrolled students will be small and only a few of them will be at the same place at the same time. Their accommodation will be arranged by the CUC in cooperation with affiliate institutions, and covered from tuition fees.

Transformative learning

Transformative learning is the expected outcome of the CUC medical curriculum. It requires students to face healthcare problems in a real setting and use the current internationally recognized concepts of successful treatment and care provision. The concept of transformative learning (Table 1) relies on changing the existing beliefs and ways

Table 1. Characteristics of the three systems of medical education*

Informative and formative learning	Transformative learning
Memorizing	Search, analysis, and synthesis of information to help with decision making
Professional acknowledgment	Acquiring key competences needed for efficient team work in healthcare system
Ready acceptance of educational models	Creative adjustment of global achievements to local priorities
Isolated systems of education and healthcare	Harmonized systems of education and healthcare
Lonely institutions	Networks, alliances, and consortiums
Strictly institutional issues	Taking over global flows of educational material, teaching options and innovations

* According to reference (1).

Table 2. Elements of medical education reform serving as the foundation for the medical school design*

Introducing a competence-based education plan.
Including competences in the processes of quickly changing local settings while relying on global sources.
Encouraging education within a profession and between professions, breaking professional chains, and stimulating cooperative and non-hierarchical relationships within teams.
Information technology use in learning.
Investing in teaching equipment and continuing teaching education.
Stimulating new types of professionalism, where competencies are taken as objective criteria for the evaluation of healthcare professionals and values built around the social accountability.
Turning academic centers into systems encompassing a network of hospitals and primary care facilities interconnected into global alliances.
Breeding the culture of critical thinking.

*According to (1).

of thinking through conversation and critical analysis in a real setting, with the aim to develop managerial competencies in active problem solving (48, 49). To fulfill this role, students and their mentors should have adequately developed professional competencies, ethical norms, and social accountability of locally accountable and globally connected teams (1). Thereby, the medical school will base its design and curriculum on the previously mentioned innovative principles, adjusting them to the current standards in defining student competencies (Table 2).

Evidence-based medicine

Evidence-based medicine (EBM) encompasses the best evidence in the literature, experience of healthcare professionals, and values and needs of patients (50, 51). For problem-solving in healthcare, one should look for evidence considered to have the highest level in the hierarchy of evidence in medicine, such as randomized controlled trials (RCTs) and systematic reviews. Results of systematic reviews are helping make informed decisions about the treatment of individual patients, making strategic and other decisions in medicine and healthcare. Not only that such scientific knowledge improves technology, but it also empowers general population. People become more informed and more aware of their healthcare needs and rights and more able to make decisions on their lifestyle changes (52, 53).

Within their EBM training, healthcare professionals should adopt the practice of *shared-decision making*, where the patient

and healthcare professionals together consider the advantages and disadvantages of available treatment options, with an aim of choosing the model of care in line with patient's wishes (54, 55).

EBM and shared decision-making will be practiced and taught at the CUC School of Medicine from the first year on, together with the traditional training in biostatistics, informatics, and research methodology in medicine, because these are the foundations of EBM. Gradually, EBM will be included in all student tasks to serve as a foundation for student's explanations, conclusions, and course of action.

Quality management and information technologies (IT)

European standards and guidelines for quality assurance in higher education area are applicable to all higher education institutions in Europe, irrespective of their organization, function, size, or national system within which they operate (56). These standards require the following:

1. A culture that recognizes and stimulates the development of quality management strategy, rules, and procedures.
2. Formal and publicly accessible mechanisms of periodical supervision of quality management system.
3. Evaluation of student knowledge and competencies in accordance with the announced criteria, rules, and procedures.
4. Availability of appropriate and competent teaching staff whose credentials may be checked by external control.
5. Availability of the equipment and conditions for studying.
6. Information system for the collection, analysis, and use of all information needed for effective management of study programs and all other related activities.
7. Regular publication of objective information on the programs (56).

Accordingly, the CUC Medical School will ensure quality management by using a business process management system that allows for designing, modeling, describing, and documenting all intra-institutional business processes, identification of responsible persons and services and related documents and software (57). Each procedure will have a clearly defined structure, process, and outcome, usually associated with data archiving (58). Such a description of business processes allows for the use of information technology in any process, and such a system of quality management allows for the use of PDCA (*plan–do–check–adjust*) approach (59) in any aspect of any work. The aim of such approach is ensuring permanent improvement and advancement according to the results and changes within an institution, and serving as a landmark and gold standard for assessment of work and quality (57).

The CUC medical curriculum

The main structure of the curriculum was developed in line with the recommendations in the Directive 2005/36/EC of the European Parliament and of the Council (60), the World Federation for Medical Education's revised standards for basic medical education (61, 62), and the global independent Commission's *Education of Health Professionals for the 21st century* (63).

The CUC medical curriculum fully implements all four recommendations from the Directive 2005/36/EC of the European Parliament and of the Council regarding the recognition of professional qualifications as of September 07, 2005 (60) and provisions of the Act on Regulated Professions and Recognition of Foreign Professional Qualifications (64) as follows: a) appropriate scientific medical knowledge and good understanding of scientific methods, including the principles of biological functions and evalu-

Table 3. Specific characteristics of the medical school curriculum

Preclinical courses are reduced and parts of teaching material related to clinical issues are integrated with clinical courses at later years.
Vertical integration of modules enabling students to approach clinical problems in a scientific manner, taking into account the principles of evidence-based medicine.
Clinical skills, as defined in clinical skill catalogue, are learned by doing (hospital internship); practical work on models starts at the first year and involves early contact with patients in family medicine offices and adequate skill catalogue.
Strongly stimulating all forms of student exchange. Mission trips will be organized for senior students to gain practical knowledge and develop a moral profile adequate for this demanding and noble profession.
Introducing students to global health topics, especially poverty-related diseases, will lead to activities aimed at reducing inequalities in access to healthcare.
The medical school will largely rely on the system of evaluation and acknowledgment of learning outcomes achieved through extracurricular and informal activities, primarily volunteering and charity work.

ation of scientifically established facts and data analysis, b) sufficient understanding of the structure, function, and behavior of the healthy and ill people and relationship between an individual's health condition and his or her physical and social setting, c) appropriate knowledge of clinical disciplines and procedures, which ensures having a complete view of mental and physical conditions, knowledge of medical prevention, diagnosis, and treatment and human reproduction, and d) appropriate clinical experience in hospitals under appropriate supervision. Among the learning outcomes, the priority will be given to the acquisition of practical knowledge and skills, which have been insufficiently represented in teaching programs of existing medical schools in Croatia (65).

Compared to the current program of medical schools in Croatia, the proposed curriculum is reduced to avoid overburdening the students with huge amounts of learning material, especially in the case of preclinical courses (Table 3). The traditional way of organizing courses in Croatian medical school assumes a division of teaching units based on the classical departments of medical schools represented by classical subjects (e.g. Anatomy, Physiology, etc.). This concept where each departments is responsible for its course, disallowed any cooperation between teachers of different departments, while the fight for the dominant position between individual departments led to unnecessary expansion of individual classes by overburdening them with unnecessary facts and consequent redundancy in course contents.

The CUC curriculum applies horizontal integration of these courses primarily based on the division into organ systems. A similar concept has been applied at Croatian medical schools only in the case of the nervous system, where the structure and function of the nervous system are integrated in the Neuroscience course. Unfortunately, no integration has been implemented for other organ systems, despite the fact that the integration in the Neuroscience course showed to be effective.

The classes will be given in Croatian or English; both languages will be equally acceptable. For students who do not speak both languages, an intensive language course will be organized. The reasons for giving lectures in English in addition to Croatian include the wider range of textbooks available in English, participation of foreign experts in teaching, and enrollment of foreign students. However, knowledge of the Croatian language is required for clinical work involving patients.

Courses

The CUC School of Medicine will last six years and include 5,500 hours of direct teaching and 360 European Credit Transfer System (ECTS) points. The classes will be organized in 10 symmetrically arranged modules (similar courses/subjects will be grouped), with each module consisting of one or more courses. There will be a total of 24 courses, including two vertical courses (Medical Humanities and Research in Biomedicine and Health) running through all six years (Figure 2). All courses will consist of 200 teaching hour, except for the vertical ones, which will consist of 275 hours. The last semester will be dedicated to research only and will result in an undergraduate thesis.

Testing student knowledge and competencies

Student knowledge in any subject area will be tested using a unified approach. The final evaluation will consist of three components: continuous evaluation during courses, practical and written exam at the end of the course. Written exams will be centralized and carried out in the form of online assessments.

Continuous evaluation will be performed using different testing modalities. At the end of each course, students will have to take an exam consisting of a practical and a written component. The final grade will be calculated according to a predefined formula, which will be identical for each exam. A practical

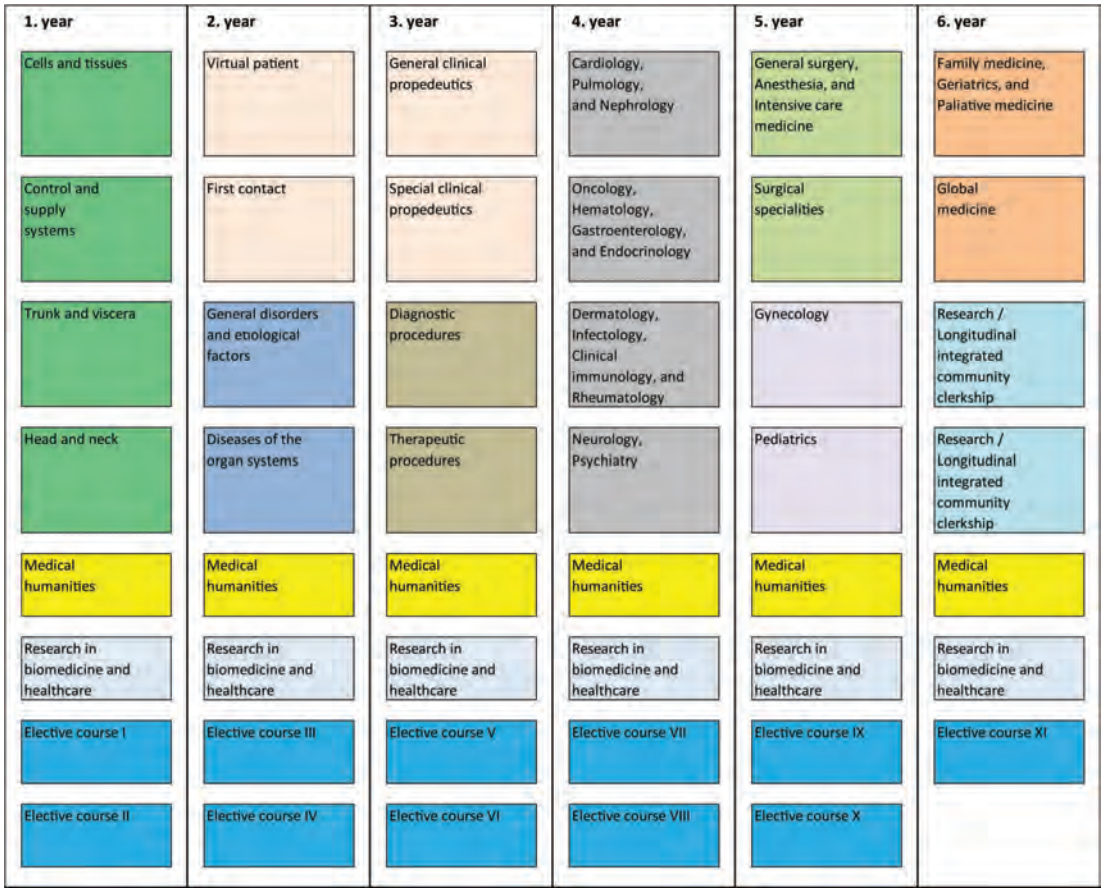

Figure 2. Organization of modules and courses taught at the medical school. Each module is color-marked and consists of several thematically-related courses. Each cell represents one course.

test as a part of the exam will be a part of courses where practical skills are relevant, and account for 30% of the grade. Written test will account for 30% of the grade and the remaining 40% of the grade will be based on continuous knowledge tested during courses. All scores will be recorded and kept in a student's e-portfolio.

Integrated exams

The purpose of integrative exams is to help students regularly reiterate what they learned (66), discourage last-minute cramming, and classify students according to their academic success (in percentiles), which will make a significant part of the *Diploma Supplement*. The Exam results also provide the feedback on the quality of teaching of individual courses, i.e., reliability of grades earned by the students.

Integrative exams include a) objective, structured, clinical examinations (*OSCE*) and b) two aggregate knowledge exams (AKE) at the end of the third and sixth year of the studies. AKE I and AKE II are multiple-choice exams consisting of 200 questions each. The number of questions from each course is proportional to the number of course hours in the respective period (67).

OSCE is an exam comprised of multipole "stations" designed to test clinical skill performance. Skills are defined in a Clinical Skills Catalogue. Stations are designed and their order organized to test the performance of each skill, or a group of simple skills, within a given period. An OSCE examination usually consists of 10 stations (67).

Graduate thesis

The work required to write an undergraduate thesis reflects a practical use of knowledge acquired during previous years of studies. A graduate thesis at the CUC School of Medicine will have to be based on original research work, including systematic reviews and meta-analyses. Students will work on their thesis-related research after having defined their research plan during the 5th year course "Research in Medicine and Healthcare".

A student will be able choose a mentor and the topic of the thesis and start with the thesis-related research as early as the first year, but will have to have a mentor and thesis topic chosen by the start of the "Research in Medicine and Healthcare" course at the fifth year. The thesis research plan will be evaluated and students/mentors will receive feedback about the plan, and if necessary, suggestions for improvement of the plan. All graduate theses will be published in English in the openly-available institutional repository.

By developing their undergraduate theses, students will acquire competencies in research methodology, collection, analysis and presentation of data, and public presentation and defense of the thesis. Students will also be trained in writing a research paper in English, selection of a suitable journal, writing a cover letter for the editor, and stimulated to send the manuscript based on a thesis to a scholarly journal. Graduate theses will be evaluated for their quality, presentation, and defense (68).

Students

The enrollment criteria will be defined in line with the social accountability of the CUC School of Medicine and needs of the society. A three-member committee independently decides on whether or not to enroll a student based on the student's high-school grades, motivational letter, curriculum vitae, and an interview. We will enroll 340 students each year, which ensures that each student receives appropriate attention and mentoring. A counseling system for students will be organized, in order to follow

their academic success, as well as their social and personal needs.

Faculty

The faculty teaching at the medical school will be divided into four groups. The core teaching faculty will be composed of experienced teachers holding academic positions, who will be employed as either full- or part-time faculty. They will be module coordinators and teach according to the CUC direct hour teaching requirements for teachers holding specific positions.

The second group are physicians working in general hospitals and other healthcare institutions in smaller communities that will participate in teaching clinical courses. They will be in charge of practical teaching and will be engaged through collaboration agreements. In Croatia, there are many healthcare institutions that are not a part of university centers but employ physicians whose professional quality does not lag behind that of their colleagues working in major university hospital centers. Although we cannot corroborate this statement with objective evidence, the opposite would imply that the healthcare system admits to the fact that population living outside larger cities is provided with healthcare of lower quality. Healthcare institutions that sign the collaboration agreement will engage an appropriate number of physicians to carry out the practical part of teaching. The third group of faculty will include young physicians who opt for academic career after graduation. They will be employed as instructors through teaching contracts. This category will serve as a potential source of future core faculty. The fourth group will include qualified Croatian physicians from abroad and foreign experts who will participate in teaching. The faculty will primarily be selected based on their excellence in academic medicine – research, teaching and practice (69).

According to the relevant legal provisions and the CUC Statute, the faculty will regularly be evaluated for their teaching, professional, and research work and results. The cooperation with external teaching sites and partner schools will be formalized through official agreements and contracts.

Research

Every higher education institution must have an adequate research activity because higher education and research cannot be separated. This is particularly important in medicine, where the triad of research, practice and teaching is the core of academic medicine. At the existing medical schools in Croatia, research has rarely been the result of a clear national, university or school strategy. The strategies that were adopted were often not implemented, and the research activity of those institution emerged as a result of the scientific interest of leading scientists in the institution, i.e. it was mainly the result of their international scientific cooperation or the result of the scientific interest of the group in which they pursued their post-graduate training. The result of such a neglect of strategic documents is the fragmentation of the scientific community in Croatia, inability to form larger research groups, poor utilization of research equipment, and poor competitiveness on the international research scene. Our Research carried out at the CUC School of Medicine will engage students as researchers and focus on research questions important for patients, public, and healthcare professionals alike (16). The first research objective of the CUC School of Medicine will be to develop an active collaboration with the Cochrane collaboration, which offers great opportunities for scientific and professional work at the international level. We hope that the collaboration with the Cochrane will help us develop methodological excellence in

research. Focus on meta-research is also important because it will also provide excellent opportunities for high quality research in conditions where the infrastructure for clinical or preclinical research is not yet developed. Finally, research on evidence synthesis can be a distributed effort, which will allow active participation of all CUC teachers/researchers regardless of their actual location (CUC premises or a local hospital).

As the CUC School of Medicine will be firmly anchored in the social science and humanities foundation of the CUC, we will focus our research activity also on social aspects of medicine, primarily on medical education and science and health policy. One of the reasons to focus on these topics is that there are many medical education, or health care policy, reforms but they are rarely systematically and rigorously evaluated. We plan to use such approach to follow and assess the impact of the new medical curriculum developed at CUC to test the value of the medical education concept presented in this article.

Conclusion

It should be mentioned that none of the programme's characteristics mentioned above that we consider worthy of describing here are original. For example, integration of subjects has already been applied by many universities. Distributed medical education is also a model used by many medical schools. However, what makes this programme original is the selection and combination of characteristics and their application in a specific Croatian context.

What is already known on this topic

Awareness of the fact that healthcare professionals receive inadequate education leads to changes in medical education. However, medical education lags behind newly emerging health needs, primarily due to the fragmented, obsolete, and static medical curricula.

What this study adds

The study presents the concept for a new medical school, based on distributed medical education, transformative learning, teaching and practicing evidence-based medicine, and implementation of quality management principles supported by information technology.

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Authors' contributions: Conception and design: DS and MMar; Acquisition, analysis and interpretation of data: DS, MMar, LP, IG, and AM; Drafting the article: DS; Revising it critically for important intellectual content: LP, IG, MM, AM, MČ, and ŽT; Approved final version of the manuscript: DS, MMar, LP, IG, MM, AM, MČ, and ŽT.

Conflict of interest: The authors declare that they have no conflict of interest.

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Continuous Assessment as a Good Motivational Tool in Medical Education

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Objective. Continuous assessment tests influence the learning needs of medical students at particular times but are also beneficial in scheduled learning, and act as motivating tool. These tests drive them to study by developing competition and optimism regarding the forthcoming result. This study has been designed to evaluate the role of these tests for medical students by learning about their motivation. **Methods.** This study was conducted on 150 medical students. The students selected a continuous testing system or a classical system. Those who selected the continuous system had six assessment tests each, after completion of a curriculum block, usually 45 days apart. The motivation of the students in both the groups was assessed by asking them for their expected results just before the final examination. Statistical analysis was performed using Superior Performance Statistical Software (SPSS) version 20. **Results.** The students' final results showed that almost half of the students who adopted the continuous assessment test system were successful and one third of those who did not adopt this system. The comparison of the successful students showed that the expectation of the students from the continuous assessment test system was high compared to the students who were not in the continuous assessment test system ($P<0.05$), even though the students in these groups had more or less similar actual marks ($P>0.05$). The comparison of the expected and actual marks of the successful students, of students who failed only one unit and of students who failed both units, showed that all these students had the same expectation ($P<0.05$) from the examination, although they had different actual marks ($P>0.05$). **Conclusion.** It was concluded that continuous assessment is good for student motivation. A support system matched with the individual needs of the students is suggested to improve their efficiency to gain the full benefits from this system.

Key words: Continuous assessment tests ▪ Medical education ▪ Learning in medical students ▪ Support system.

Introduction

Medical students are the product of diverse systems and educational backgrounds, and have different hopes, standards, learning styles, adaptive skills and coping mechanisms (1). These influences and their motivation during their time at medical college have an important role in their learning, and drive them to perform in their professional examinations. Intervention in good time is important to improve the success rate and retention of at-risk students. Research in various settings has consistently shown that perception of high self-efficacy is an important positive motivational belief (2). Motivational scales are based on a broad so-

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cial-cognitive model of motivation that proposes three general motivational constructs, which are: expectancy, value and affect (3).

This study was conducted to discover student motivations, measured through their expectations after continuous and periodic tests.

Methods

This study was conducted from Jan, 2015 until March, 2016 at Karachi Medical and Dental College, after acquiring approval from the institutional ethical committee (Ref; 453/15/Ph). One hundred and fifty undergraduate medical students were included who were enrolled in the pharmacology course as a requisite of the 3rd year MBBS curriculum of the Pakistan Medical and Dental Council. The study was explained to the students and consent was obtained by e mail, using the official ID of the investigator. Ninety-five students adopted the continuous assessment test system, while fifty five students did not adopt this system. All students attended lectures at the same faculty, followed the same syllabus, and were taught under the same conditions. Students who adopted the continuous assessment test system took six tests, each of which was forty-five days after the previous test, upon completion of a certain stage of teaching and training, over nine months, and the result of each test was published within one week. Only students were allowed to sit the final examination who had attended 75% or more teaching sessions, and who had taken any four of the six tests throughout the term, in the case of those students who adopted the continuous assessment test system.

The final examination contained two sections, one for assessment of theoretical knowledge and the other for examining the students' practical knowledge. Each student was asked to write his expected marks on a paper just before the examination. These marks were kept confidential. Access to them was provided to the investigator after the final result.

The students were divided into three groups on the basis of their official results. The first group consisted of students who had passed the examination and attained equal to or more than 50% of marks in both sections. The second group comprised those students who failed one section and they all attained less than 50% of the marks from one of the sections, and the third group comprised those students who failed both sections and attained less than 50% of the marks from both sections separately.

Statistical analysis

We present the individual responses in tables. Distribution of the continuous variables was expressed as the mean and standard deviation, and compared by Fisher's exact test. Statistical significance was confirmed at $P<0.05$. We used the statistical analysis software package SPSS 20 (SPSS Inc., Chicago, Illinois, USA) for data processing.

Results

Among the total of 150 students, 95 (63.3%) adopted the continuous assessment test system while 55 (36.6%) did not participate in this system. The students' final results show that 53 (55.7%) from the group of students who adopted the continuous assessment test system and 19 (34.5%) from the group of who did not adopt this system, passed the examination.

The mean of the expected marks of the successful students in the continuous assessment test system was 62.9 and of the students who were not in the continuous assessment test system it was 48.1. The difference is statistically significant ($P<0.05$). The mean of the actual marks of the successful students in the continuous assessment test

Table 1. Comparison of expected and actual marks of the successful students in the two groups

Type of marks	Students in the continuous assessment test system		Students not in the continuous assessment test system		P*
	N	Mean ±SD	N	Mean ±SD	
Expected	53	62.9±06.8	53	48.1± 08.1	0.021
Actual	19	63.7± 05.5	19	61.1± 06.5	0.237

*Fisher's exact test.

Table 2. Differences between the actual and expected marks of the different groups of students.

Group	Students (N)	Marks obtained (%)*	Expected marks (%)*	Difference
Pass	53	63.7	62.9	00.7
Failed one section	22	46.2	55.1	08.9
Failed both section	20	34.4	53.5	19.1

*Mean of % marks.

system was 63.7 and of the students who were not in the continuous assessment test system it was 61.1. The difference is not statistically significant (P>0.05) (Table 1).

Comparison of the two shows that the expectation of the students in the continuous assessment test system was quite high as compared to the students who were not in the continuous assessment test system, even though students from both groups had more or less similar actual marks. The difference between the actual and the expected marks of the different groups of students increases as failure occurs. It was 00.7 for successful students, 08.9 for students who failed one section, and 19.1 for students who failed both sections (Table 2).

Discussion

Medical schools prepare professionals who take on important responsibilities in human society. These schools increasingly want to understand better why some students have difficulty passing medical courses. These studies can offer medical schools an in-depth insight into how they might positively influence overall student performance (3-5). We considered the role of continuous assessment tests in this regard. Although these tests influence personal learning preferences at particular times (6), they also help in the successful management of the curriculum by systematic assessment (7) and scheduled learning activities (8). We found that the group of students who adopted the continuous assessment tests system showed better results as compared to those who did not adopt this system.

A constant challenge is to motivate students entering medical college who are less well prepared for the demands of their future student life, psychologically, socially, and academically. Successful remedial and study strategy courses aimed at underprepared students have demonstrated that students who really want to improve their skills can do so when motivated (9). Placing the appropriate emphasis on testing and grading is considered to be important in the strategies, and they are needed to motivate students (10). We consider that continuous assessment tests develop competition in medical students, who are strategic learners and react well to competition. These tests are satisfying because they help develop a feeling of being in control (11) and play an important role in building their optimism (12). We, therefore, used this tool as a motivation agent in this study.

Motivation can be assessed by expectations (13). It plays a major role in their progress by encouraging efforts to reach a target (14). We found that the group of successful students who adopted the continuous assessment tests system showed high expected marks as compared to those who did not adopt this system. The mean of the expected marks of the students in the continuous test system was significantly higher than the mean of the students in the other group, who were not in this system.

All the students in the continuous assessment test system participated in the same activities, passed through the identical teaching environment and attended tests equally, so we expected that they had more or less the same motivating influences. Perhaps this may be a reason why all the students in this group shared the same expectations for their results, as is clear from a comparison of the mean of the expected marks of the students who passed, or those who failed one section or failed both sections. Their means are not statistically different.

Although all the students in the continuous assessment test system received identical motivation and developed similar expectations, a number of students in our study could not pass the examinations. This fact leads to consideration of the factors which influence a student's performance. Some factors have an exogenous origin. The importance of resource management strategies has been reported by a number of researchers (12, 15). Our study did not focus on exploring these factors. The interwoven system of the structure of student needs, the teachers and their teaching abilities, and the environmental dynamics, control the factors in which they spend most their time. Throughout the world, medical educators are attempting to reform the educational environment to make it more student-friendly, without compromising the standards and quality of learning (16). The application of heutagogical principles in undergraduate medical education has not yet been studied extensively (17).

Other factors have an endogenous origin and are directly related to the student's health. Depression may be one of the causes which prevent many students from learning and performing in examinations. Roughly a third of college students report feeling depressed (18). This figure is substantially higher than in the general population (19) and appears to be increasing (20). Perhaps goals related to extrinsic values (money, image, fame) have become more important and those related to intrinsic values (self-acceptance, affiliation, community) less important nowadays, and this may also be a cause of the decline in performance (21-23).

Medical students are at high risk for depression and suicidal tendencies. In a recently published systematic review, it was estimated that 27.2% of medical students display depression or depressive symptoms, and 11.1% suicidal tendencies (24), which is a very alarming threat for medical education. Literature identifies some interventions which have been found to control this tendency (25). Friends play a significant role in mental and physical health (26). Considering all the factors of exogenous or endogenous origin, it appears that a support system must be developed in a continuous assessment test setting, which can provide help for students in coping with their deficiencies. Without this system the benefit of motivation and therefore, of the continuous system, cannot be achieved. In a study by Eccles, et al., a decline in academic performance is a reliable predictor of low self-concept, intrinsic motivation, and confidence in intellectual abilities. It is proposed by these researchers that such declines result from a developmental mismatch between early adolescents and their classroom environment, which in turn results in negative motivational outcomes, especially for struggling students (27).

Conclusion

Our study suggests consideration by other investigators of other factors, such as fear or obligation, or a sense of responsibility, which may also have been increased by the continuous testing system, to explore the role of this system in medical students in order to optimize its benefits.

What is already known on this topic

Continuous Assessment is the educational strategy in which students are examined continuously during their study. It is a method of observing and collecting information periodically to find out what a student knows and understands. Various studies have proved the worth of this system as a tool for transmission of knowledge, and organization of academic activities, skills and abilities. However, its role in motivating students or encouraging students to acquire knowledge is perplexing, so clarification is necessary to optimize its benefits.

What this study adds

Our research broadens the understanding of the importance of the continuous test system and supports the concept that it is not merely a tool of assessment of students but also produces motivation in them to study.

Authors' contributions: Conception and design: MTA and MHT; Acquisition, analysis and interpretation of data: MHT; Drafting the article: MTA; Revising it critically for important intellectual content: MTA and MHT; Approved final version of the manuscript: MTA and MHT.

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Case report
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Complete Response of Metastatic Melanoma to Second Line Chemotherapy with Paclitaxel and Carboplatin – Case Report

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Key words: Metastatic melanoma ▪ Chemotherapy ▪ Second line.

Objective. We present a patient with metastatic melanoma who had a complete response to second line chemotherapy with paclitaxel and carboplatin. **Case report.** Metastatic melanoma is an aggressive cancer with poor prognosis and 10 year survival less than 10%. We present a patient with metastatic melanoma who had a complete response to second line chemotherapy with paclitaxel and carboplatin. **Conclusion.** Second line chemotherapy with paclitaxel and carboplatin in the treatment of metastatic melanoma may yield effective results.

Introduction

Melanoma is an aggressive tumour with the advanced disease characterized by widespread metastatic lesions, and the tumour has traditionally been resistant to most forms of treatment. Median survival for metastatic disease is only 6 – 9 months, a 1-year survival rate of 25%, and a 3 year survival of 15% (1).

The incidence of malignant melanoma in Europe varies from 19.2% in Switzerland to 2.2% in Greece per 100,000 persons/year (2). Most patients with metastatic melanoma need systemic treatment. Metastatic melanoma is considered to be a chemotherapy resistant disease so the role of chemotherapy in stage IV is less significant. Two distinct therapeutic modalities, tyrosine kinase inhibitors and immunotherapy might improve response rate, progression free survival and overall survival for metastatic melanoma (1). In many transition countries, such as Bosnia and Herzegovina, institutional barriers and the poor socioeconomic situation make it difficult to provide cancer treatment according to contemporary guidelines, and we are forced to treat patients with the available chemotherapy drugs.

In this case report we showed that chemotherapy can achieve excellent results in the treatment of metastatic melanoma.

Case report

A 57-year old female patient with ECOG (Eastern Cooperative Oncology Group) performance status 0, was diagnosed with malignant melanoma of the trunk in July 2011. In terms of comorbidities, she had arterial hypertension, atrial fibrillation, coxarthrosis and gonarthrosis of the left knee.

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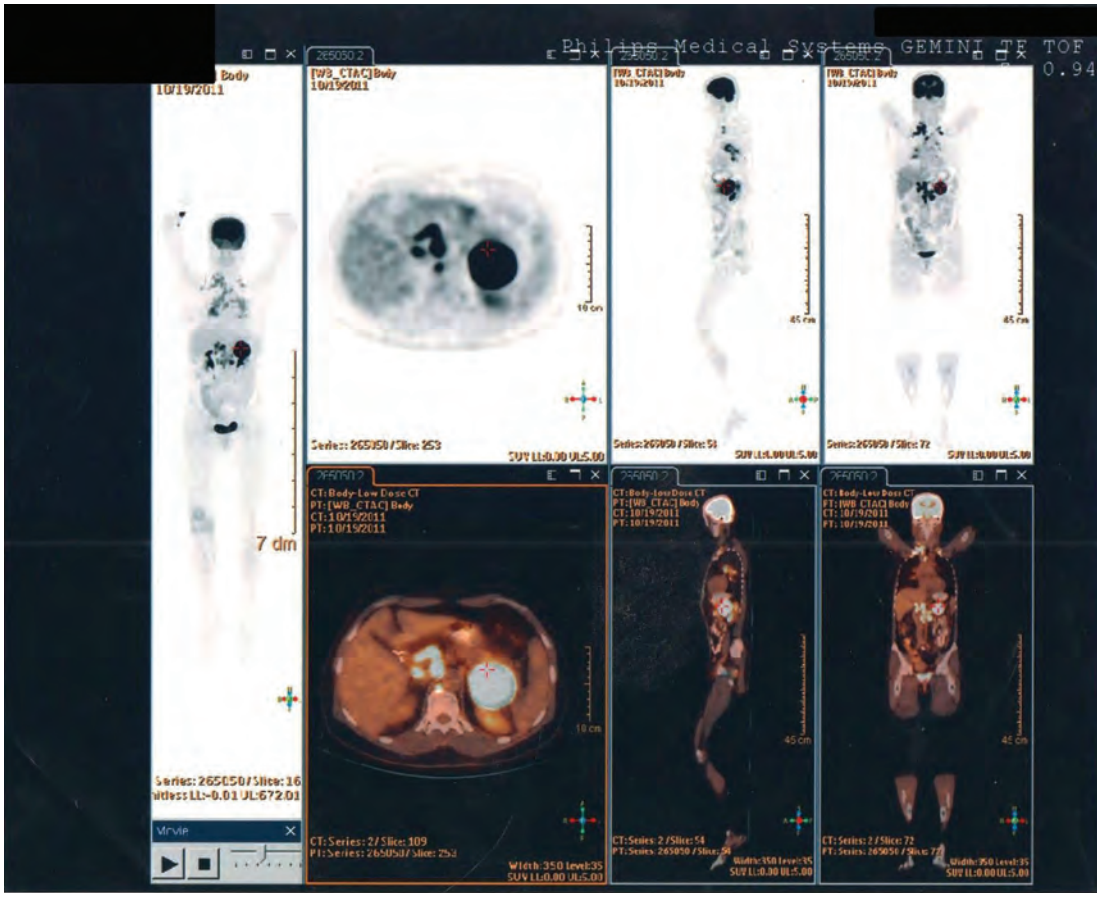

Figure 1. PET/CT scan; pathological metabolism of glucose in the lymph nodes of neck, mediastinum and abdomen, lesions in the pulmonary parenchyma and left adrenal gland.

The pathology report after surgery showed a malignant melanoma, Clark 4, Breslow 4. BRAF V600E analysis was not initially performed, but subsequent analysis confirmed BRAF V600E mutation. Postoperative PET/CT in October 2011 detected metastatic melanoma in the lymph nodes of the neck, mediastinum, abdomen, and lesions in the pulmonary parenchyma and the left adrenal gland (Figure 1).

The serum LDH level was within reference range. Fine needle aspiration cytology (FNAC) of the lymph node at the neck did not show malignant cells. At the end of October 2011, chemotherapy was initiated with dacarbazine (250 mg/m2/day) and it was given intravenously for 5 days every 4 weeks. After 3 cycles of chemotherapy, control CT scans showed the stable disease by RECIST criteria, but after 7 cycles of chemotherapy control PET/CT showed progression of the disease with newly discovered metastases in the bones. From the side effects of dacarbazine chemotherapy the patient had grade 1 anaemia and grade 1 nausea. Chemotherapy with dacarbazine was discontinued due to the disease progression in May 2012, and we then started second line chemo therapy treatment with paclitaxel (175 mg/m2) and carboplatin (AUC 4) (TC regimen) intravenously every 3 weeks. Bisphosphonate Zolendronic acid was administered every 4 weeks because of the bone metastasis. Regular follow up with MSCT scans and PET/CT was performed during treatment. Due to peripheral neuropathy grade 3 we reduced

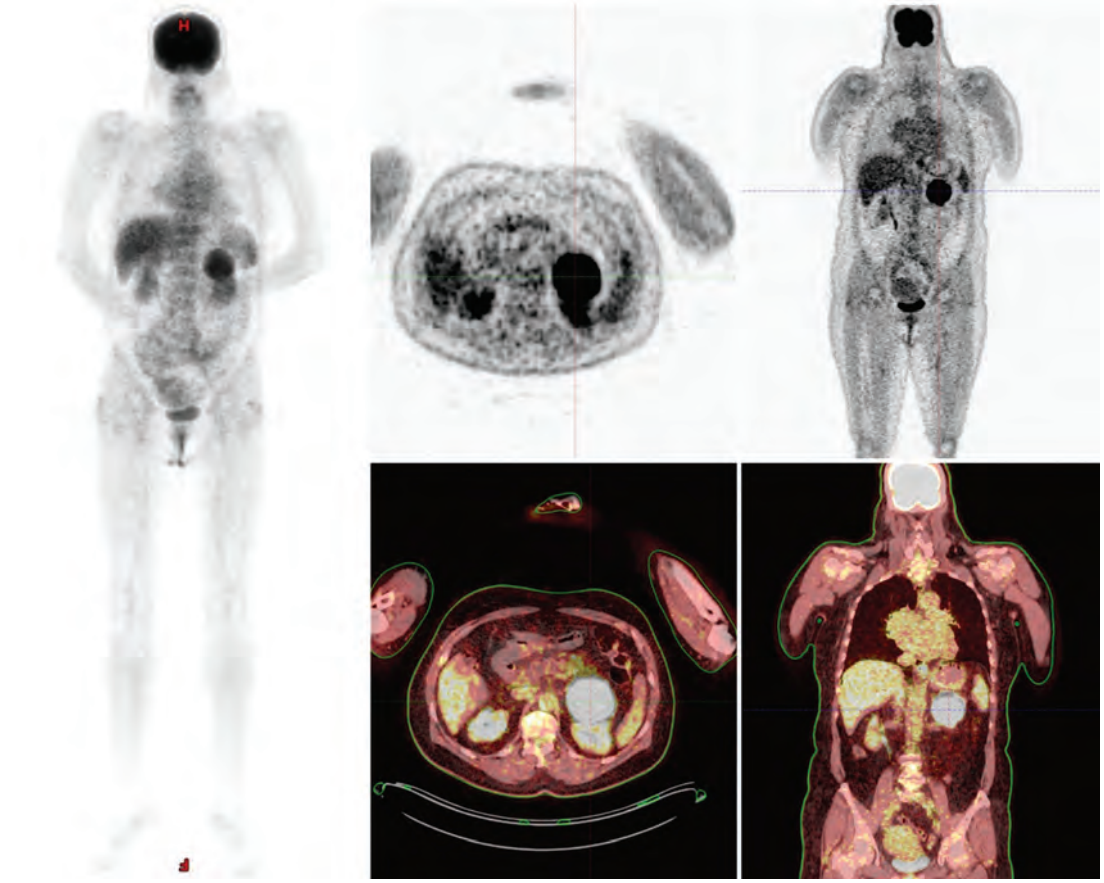

Figure 2. PET/CT scan; pathological metabolism only in left adrenal gland.

the dose of paclitaxel by 20% after 11 cycles of chemotherapy. Other side effects were grade 2 leukopenia, grade 2 granulocytopenia, grade 1 anaemia, grade 1 hypokalaemia and grade 1 hypocalcaemia. After 22 cycles of chemotherapy, in January 2014, control PET/CT showed complete disease response with a stable lesion by RECIST criteria in the left adrenal gland (Figure 2).

In March 2014, left adrenalectomy was performed and the pathology report showed a 5 centimeter benign granulocyte cell tumour. After that, a control CT scan showed no evidence of disease, so we discontinued all oncological treatment and continued regular follow-up. The last oncologist examination was in January 2016. The patient was in good general condition and the control CT scan showed no evidence of disease. In April 2016 we found by heteroanamnesis that the performance status of the patient was poor, and from the accompanying medical documentation we could see that the patient had suspected disease progression on the pleura, liver, kidney and tumour formation with heart compression. The patient died in May 2016, and the cause of death was probably related to disease progression.

Discussion

We report complete response to second line chemotherapy with TC regimen in a patient with metastatic melanoma. The treatment goal for metastatic melanoma patients is to prolong overall survival, reduce tumour-related symptoms and maintain quality of life. The treatment of metastatic melanoma

has undergone huge changes in the last few years. Before the era of new therapeutic options, we treated patients with chemotherapy, such as dacarbazine, temozolamide, paclitaxel, albumin-bound paclitaxel, and carboplatin/paclitaxel or docetaxel (3).

According to previous data, dacarbazine showed an improved objective response rate (ORR), median progression free survival (PFS) and overall survival (OS) (4, 5). Our patient had PFS for 5.5 months with Dacarbazine, the best treatment response was a stable disease with side effects such as grade 1 anaemia and grade 1 nausea, which was similar to the reported side effects in the trials with Dacarbazine (4, 5). There have been two phase 2 studies that showed positive results with taxane and carboplatin in the second line treatment of metastatic melanoma (6, 7), with one complete response with docetaxel and carboplatin (7). Also there have been two phase 3 studies that showed no statistically significant difference in PFS, OS and ORR, when comparing the paclitaxel and carboplatin regimen (TC) with or without sorafenib in first and second line metastatic melanoma (8, 9). In study by Pflugfelder A, et al. (10) which compared the effectiveness of the TC regimen in the first and second line treatment of metastatic melanoma, the results were equivalent in PFS, OS and response.

Here, we present an isolated case of a patient with extensive chemotherapy duration and complete response to a paclitaxel and carboplatin regimen. The optimal duration of chemotherapy in metastatic melanoma has not been defined. In clinical practice we continue chemotherapy treatment until disease progression and/or unacceptable toxicities. In comparison to the other studies, it is important to notice that our patient had a complete response after 22 cycles of second line chemotherapy with the TC regimen (6, 8, 10). In the study by Lee CK, et al. (7) there was one complete response to second line chemotherapy with docetaxel and carboplatin, which is similar to our results with paclitaxel and carboplatin. Due to grade 3 peripheral neuropathy during treatment with the second line TC regimen, we reduced the dose of paclitaxel by 20% after 11 cycles of chemotherapy. Other side effects were grade 2 leukopenia, grade 2 granulocytopenia, grade 1 anaemia, grade 1 hypokalaemia and grade 1 hypocalcaemia. Our patient´s adverse events corresponded to the adverse events verified in the phase 2 and 3 studies (6, 8, 9, 7).

For a BRAF V600E mutated metastatic melanoma, targeted therapy with tyrosine kinase inhibitors vemurafenib and dabrafenib showed significantly better results in first line treatment, compared with Dacarbazine (11, 12). The combination of vemurafenib and cobimetinib compared to vemurafenib has statistically significantly greater PFS and ORR, at the cost of increased toxicity (13), and the combination of dabrafenib and trametinib, compared to dabrafenib, has statistically significantly greater PFS and OS (14).

Immunotherapy is a new therapeutic option that has shown excellent results in the treatment of patients with metastatic melanoma. The anti PD-1 antibodies nivolumab and pembrolizumab, anti CTLA antibodies ipilimumab and the combination of nivolumab and ipilimumab are immunotherapy options used in metastatic melanoma patients (3). A recently published phase 3 study showed the superiority of nivolumab and ipilimumab compared to monotherapy with nivolumab or ipilimumab. The median OS was not reached in the combined group, versus 37.6 months in the nivolumab group, which was statistically significant compared to 20 months with ipilimumab after 36 months of monitoring (15). The overall survival (OS) of our patient was 55 months, which was a significant result, comparable to the results of new therapeutic options, and significant in comparison to survival

achieved by chemotherapy in randomized clinical trials (11-14, 15).

Since Bosnia and Herzegovina belongs to the group of countries with low socioeconomic development, new therapeutic options are unavailable because of their high prices. According to the recently published study by Kandolf-Sekulovic, et al. (16), more than 5000 patients in Europe do not have access to the recommended first line therapy for treatment of metastatic melanoma. With the lack of new therapeutic options, such as immunotherapy and targeted therapy, our patient case report shows that there are isolated cases where excellent therapeutic results can be achieved with the available therapeutic options, such as paclitaxel and carboplatin.

Conclusion

Treatment of metastatic melanoma with chemotherapy has inferior results compared to new therapeutic options, including tyrosine kinase inhibitors and immunotherapy. Due to the unavailability of new therapeutic options, chemotherapy represents the only available therapeutic modality for most patients in Bosnia and Herzegovina. Our case report showed that chemotherapy can achieve excellent results in the treatment of metastatic melanoma and this case could be interesting for other countries with similar local situation and not only for Bosnia and Herzegovina.

What is already known on this topic

Standard treatments of metastatic melanoma according to oncology guidelines are tyrosine kinase inhibitors and immunotherapy. Chemotherapy produces modest results in the treatment of metastatic melanoma.

What this study adds

Due to the unavailability of new therapeutic options, chemotherapy represents the only available therapeutic modality for most patients in Bosnia and Herzegovina. We report complete response in our patient which is similar to study by Lee CK, et al. about the potential clinical activity of second line combination treatment with taxane and carboplatin in metastatic melanoma (7).

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Conflict of interest: The authors declare that they have no conflict of interest.

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Case report
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Lymphovenous Anastomoses Between Thoracic Duct and Azygos Vein in a Human Cadaver: A Case Report

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Objective. The study adds valuable information regarding lymphovenous communications between the thoracic duct and the azygos vein, which are very rarely discovered during anatomical dissections and very few cases have been mentioned worldwide. A detailed description of our findings and a brief review of the relevant literature are also provided. **Case report.** In the current study, two sizeable obliquely directed lymphovenous anastomoses between the thoracic duct and the azygos vein at the midportion of the mediastinum are described in the same cadaver. **Conclusion.** The existence of such anastomoses in humans, as well in animals, is a scientific issue under debate. Cases of rapid cancer spread could be potentially explained by the likely presence of the abovementioned communications.

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Key words: Lymphovenous anastomoses ▪ Thoracic duct ▪ Azygos vein.

Introduction

Lymphovenous anastomoses (LAs) in humans were described by Frantschi in 1948, who described communications between veins and lymphatics, especially at the height of the 12th thoracic and the 2nd lumbar vertebra, mostly connected with the system of the vena cava. Frantschi claimed that these anastomoses are of regular occurrence in humans too, since he had perfected his dissection technique and concluded that they exist in all cases, without exception (1). In contrast, post Frantschi researchers, such as Zhdanov (1952) and Griaznova (1953), strongly disagreed with the existence of the so-called "anastomoses of Frantschi" after meticulous dissection of a large number of human cadavers (1, 2). However, the presence of LAs has been proposed to protect the development of postmastectomy lymphedema and could also explain cases of rapid metastatic tumor evolution.

In the current study, LAs between the thoracic duct and the azygos vein are depicted and described, and a short review of the literature is provided in order for the reader to comprehend the anatomy, physiology, embryology and clinical applications of such communications.

Case report

During a routine dissection in our Department of Anatomy and Surgical Anatomy, we encountered LAs between the thoracic duct and the azygos venous system. The dis-

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section was conducted on an 80-year-old formalin-fixed male cadaver, used for educational and research purposes, whose death was unrelated to the present case report. Specifically, after meticulous dissection of the thorax region and the mediastinum, and after the excision of both lungs and the heart by means of the classical method of anatomical dissection, we detected LAs between the thoracic duct and the azygos vein. The specific cadaver displayed azygos, hemiazygos and accessory hemiazygos veins in the expected course in the mediastinum, whereas the thoracic duct was detected ascending normally through the posterior mediastinum between the azygos vein and the thoracic aorta. In particular, at the level of the fifth and sixth thoracic vertebra, two sizeable veins arising from the azygos vein were directed obliquely to the trunk of the thoracic duct. The distance between the two LAs was 2.1 cm, whereas the diameter of each LA was approximately 1.2 mm (Figure 1). Our findings were documented by several photographs taken using a Nikon D3100 digital camera, and the measurements were made using a digital vernier caliper with an accuracy of 0.01mm. No other congenital anomalies, variations or pathological conditions, or evidence of previous surgical interventions in the region were present.

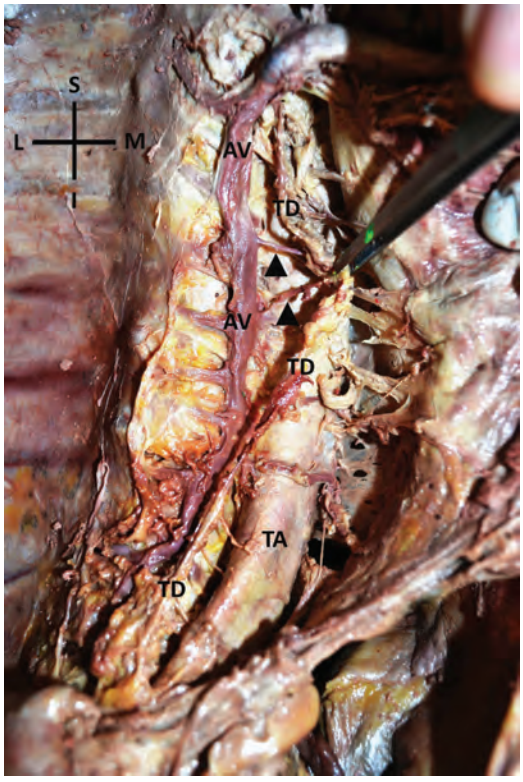

Figure 1. The azygos vein (AV) along with the thoracic duct (TD) in the posterior mediastinum are demonstrated communicating with two obliquely directed sizeable branches, the so-called lymphovenous anastomoses (arrowheads) (TA: thoracic aorta, S: superior, I: inferior, L: lateral, M: medial).

Discussion

The origin of both azygos and hemiazygos veins is in the union of the ascending lumbar veins and the subcostal veins. The azygos vein ascends in the posterior mediastinum after it passes through the diaphragm, and terminates at the level of the fourth thoracic vertebra, arching anteriorly to the superior vena cava. The tributaries of the azygos vein are the posterior intercostal, bronchial, esophageal, pericardial and mediastinal veins (3). In contrast, both hemiazygos and accessory hemiazygos veins lie on the left side of the vertebral column in the mediastinum. Specifically, the first one begins, like the azygos, on the left, ascending to the eighth thoracic level, where it ends in the azygos vein. Tributaries of the hemiazygos are the lower three posterior intercostal veins, the esophageal and mediastinal branches, the left ascending lumbar vein, and the subcostal vein. The accessory hemiazygos vein receives blood from the fourth to eighth intercostals, the left bronchial veins, and often joins the hemiazygos and ends in the azygos vein (4).

The thoracic duct is a continuation of the cisterna chyli above the diaphragm, which lies between the abdominal aorta and the right crus of the diaphragm, and receives lymph from the abdomen and the

lower limbs (5). The duct travels through the posterior mediastinum between the azygos vein and the thoracic aorta, and reaches the level of the sixth to fourth thoracic vertebra, where it crosses to the left side and extends to the root of the neck (6). It terminates at the junction of the left internal jugular vein and left subclavian vein at the base of the neck, receiving lymph from the left jugular and subclavian lymph trunks, in order to drain all the lymph of the body, apart from the right arm and the right halves of the thorax, head and neck (7).

The embryological origin of the azygos venous system could clarify the existence of such variations in the region, as in our study. The first network of lymphatic capillaries is dispensed along the primary, cardinal venous trunks. Six lymph sacs, which are paired, that is the jugular sacs, retroperitoneal sacs and posterior sacs, develop and consist of blood which is discharged into the nearby veins, thereby losing its vein communications. Down-growths from the jugular sacs unite with each other and meet upgrowths from the cisterna chyli, to produce the thoracic duct, which links the several sacs into a common system. The jugular sacs are the only ones to develop permanent connections with the vein system. Finally, the various sacs themselves break up into networks and are mainly replaced by chains of lymph glands (8). The abovementioned permanent connections between the jugular sacs and the veins could explain the LAs between the thoracic duct and the azygos vein, as in our case.

We consider that our reported case is exceptional since such a communication between the azygos vein and the thoracic duct is mentioned extremely rarely in the literature. To the best of our knowledge, such a case of a communication between the system of azygos veins and the thoracic duct was only mentioned recently by Dahran and Soames (9). In their study, these authors came across two branches of the thoracic duct, which drained into the accessory hemiazygos vein at the level of the eighth and ninth thoracic vertebra. Moreover, these tributaries were extremely small, less than 1 mm in diameter. In contrast, in our study the two LAs were between the thoracic duct and the azygos vein at the level of the fifth and sixth thoracic vertebra, while their size was approximately 1.2 mm in diameter. Van Limborgh observed these LAs in the thoracic prevertebral region in 50% of his cadaveric material, and concluded that these vessels are not true LAs, but constitute "vasa lymphatica vasorum" (10). Similarly, Griaznova, after perfusion of a gelatinous substance inside the thoracic duct in 10 human cadavers, was not able to detect any LA (2). It has been proposed that the normal presence of LAs in the upper limb may act as protection from postmastectomy lymphedema, since these vessels allow adequate lymph flow drainage from the arm, after radical excision of axillary lymph nodes (11). It has been shown that these LAs are more frequent in animals, and particularly in dogs. Specifically, de Freitas et al. observed such LAs between the thoracic duct and the azygous vein system in 60% of examined dogs, following ligature of the duct. However, under normal conditions, those authors failed to notice such LAs (12).

Thus, it may be suggested that these LAs could explain cases of rapid cancer spread from the lower part of the body, such as cancer of the gastrointestinal tract or pelvis, and lower limb tumors, to the lungs or other organs (9). Since the LAs in our study consist of sizeable vessels, it may be proposed that the hematogenous distribution of cancer cells from the lymph nodes to the arterial circulation is not only efficacious, but a short path is provided for rapid metastatic tumor evolution.

Conclusion

Very few cases of LAs between the thoracic duct and the azygos vein have been recorded and published in the literature. Specifically, a double azygos-thoracic duct anastomosis is described in the current study, and could be an asset to present-day physicians to find an explanation and finally treat cases of rapid cancer spread. Moreover, the awareness of such LAs may be helpful for surgeons in the region when they come across vessels of an unknown nature, such as an anastomosis of the thoracic duct with the azygos vein.

What is already known on this topic

The existence of communications between the thoracic duct and the azygos vein has been a topic under discussion for many years, and they are considered to be extremely rare. Most researchers in the past, after dissecting a sufficient number of human cadavers, came to the conclusion that such communications do not exist in humans, but only in dogs.

What this study adds

This study adds important information regarding this very rare variation, that has been mentioned by very few researchers in the literature. A photograph of the case and an analytical description of the dissection are provided and a short review of the literature is given. The anastomosis between the thoracic duct and the azygos vein probably has embryological origin. Clinical considerations, explaining cases of rapid cancer spread, may arise for physicians.

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Authors' contributions: Conception and design: KK and GP; Acquisition, analysis and interpretation of data: KK, GP and MP; Drafting the article: KK, GP, GN and KN; Revising it critically for important intellectual content: KK, GP and GN; Approved final version of the manuscript: KK, GP, MP, GN and KN.

Conflict of interest: The authors declare that they have no conflict of interest.

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Case report

Images in clinical medicine
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Spontaneous Intraparenchymal Lung Haematoma with Active Bleeding, Associated with *S. Aureus*

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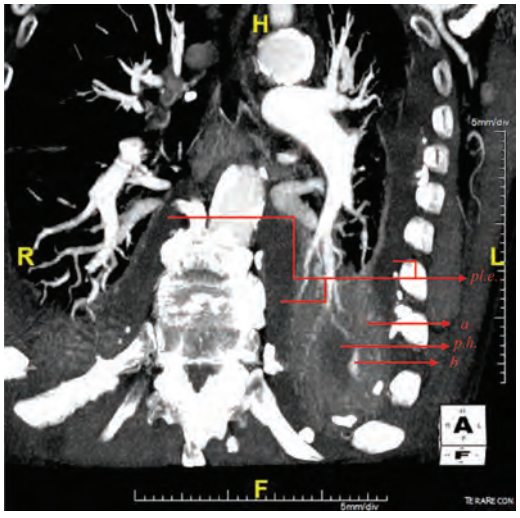

Figure 1. Oblique inferior coronal projection. a=Atelectasis; b=Active bleeding; p.h.=Pulmonary hematoma; pl.e.=Pleural effusion.

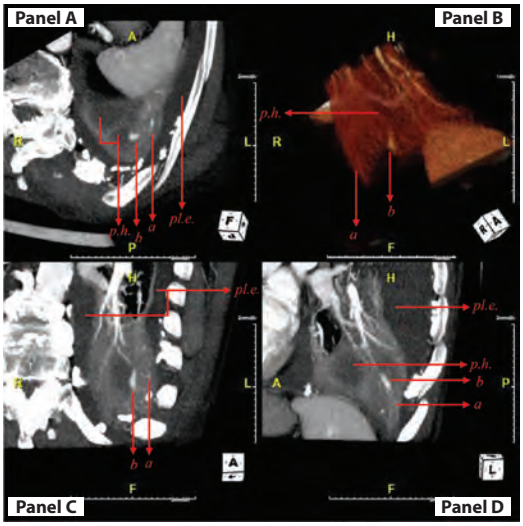

Figure 2. Comparison of oblique projections. Panel A: oblique left posterior transverse planes; Panel B: oblique right inferior coronal plane. 3D-volume-rendered vascular distribution in left lower lobe CT-angiography; Panel C: oblique inferior coronal projection. Lower left lobe detail; Panel D: oblique anterior superior left sagittal plane.

A 91-year-old patient with hypertension and chronic kidney impairment was evaluated for dyspnoea and haemoptysis in a peripheral hospital. The patient appeared pale, dyspnoeic, and tachycardic, but normotensive, presenting with a vesicular murmur reduction in the lower left pulmonary field, normocytic anaemia (haemoglobin 8.6 g/dL), white blood cell count: 5.68x10^3/µL, C-reactive protein: 26.4 mg/L, moderate renal impairment and normal coagulation tests (INR 1.15, APTT 27.9 s). A left lower lobe pulmonary hematoma (4.8 cm) with active bleeding (Figure 1) and pleural effusion with partial atelectasis were found on the chest CT-angiography (Figure 2). Further, some enlarged mediastinal lymph nodes were evident, as well as diffuse emphysema [not shown, *A/N*]. The patient was transfused and sent to a hospital with inter-

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ventional radiology; however neither embolization nor thoracic surgery were attempted because of the peripheral localization of the bleeding and the patient's clinical features. Conservative treatment and antibiotics permitted clinical and laboratory improvement. Quantiferon and the Venereal Disease Research Laboratory excluded tuberculosis or *T. pallidum* infection; a bronchoscopy with bronchoalveolar lavage and transbronchial needle aspiration was performed, showing no evidence of malignancy; neutrophils and positive culture tests suggested acute *S. aureus* respiratory infection. The diagnosis of spontaneous pulmonary hematoma with intraparenchymal haemorrhage was made, as no history of trauma was reported and we could not find any known risk factors. Parenchymal lung hematomas presenting with haemorrhage are rare, life-threatening conditions, associated with trauma, chest surgery, cancer, tuberculosis, vascular malformations and anticoagulation. Only a few cases of spontaneous idiopathic pulmonary hematomas have been reported, all showing parenchymal lung disorders, such as chronic obstructive pulmonary disease (1), isolated bronchiectasis (2) or emphysema (3), suggesting that primary lung diseases may underlie spontaneous pulmonary haemorrhage. However, all these patients had some possible risk factors, such as the use of platelet aggregation inhibition therapy or steroid drugs. None of these conditions was present in our patient. Probably the precipitating factor in our case of parenchymal lung haemorrhage was bronchitis, although it has never been reported as an isolated risk factor for spontaneous intraparenchymal lung hematoma with haemorrhage.

Key words: Lung haemorrhage ▪ Lung bleeding ▪ Haemoptysis.

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Historical article

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Medical Journals in Bosnia and Herzegovina from 1878 to 1945

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Key words: Medicine ▪ Journal ▪ Bosnia and Herzegovina ▪ 1878-1945.

This paper presents the medical journals published in Bosnia and Herzegovina (BIH) in the period from 1878 to 1945. The first medical journal in BIH may be deemed to be *Jahrbuch des Bosnisch-Hercegowinischen Landesspittales in Sarajevo* (The Yearbook of the National Hospital of Bosnia and Herzegovina in Sarajevo). In a special part of this journal, doctors from Austro-Hungary serving in Sarajevo wrote scholarly articles about their patients' various ailments. Up to 1945 seven more medical journals were published in BIH: *Trezvenost – Organ J*ugoslavenskog Saveza Trezvenosti (Temperance – the Journal of the Yugoslav Temperance Society), *Zdravlje – Lekarske pouke o zdravlju i bolesti* (Health, Medical lessons on Health and Disease), *Glasnik Lekarske komore za Bosnu, Hercegovinu, Dalmaciju i Crnu Goru* (The Journal of the Chamber of Physicians of Bosnia, Herzegovina, Dalmatia and Montenegro) *Glasnik Lekarske komore Vrbaske banovine* (Journal of the Chamber of Physicians of Vrbaska banovina, *Glasnik Lekarske komore Drinske banovine* (Journal of the Chamber of Physicians of Drinska banovina), *Vjesnik Zavoda za suzbijanje endemijskog sifilisa u Bosni i Hercegovini* (Journal of the Institute for Combatting Endemic Syphilis in Bosnia and Herzegovina) and Časopis za medicinu i biologiju (Journal for Medicine and Biology). **Conclusion.** Medical journals published in BIH in the period from 1878 to 1945 were published in times marked by specific political and social circumstances in BIH, in the time when BIH was not independent, and was under the influence of the health culture of the ruling regimes. Most of the authors of the articles published in these journals were citizens of the occupying authorities, although the papers published were mainly the result of research undertaken in BIH.

Introduction

Journals have been the basic medium of professional and scholarly communication for more than three centuries. The first scientific journal in the world, *Journal des sçavans*, was published on 5 January 1665 (1) and, only fourteen years later, at the beginning of 1679, the first general medical journal was published, entitled *Les Nouvelles descouvertes sur toutes les parties de la médecine* (2). Specialized medical journals were not published until the beginning of the 20th century. Already from the beginning of publication of medical journals, they became the main link between medical science and practice. The launch of a medical journal and its continued publication are closely linked with the overall social circumstances and the degree of development of the medical profession and science in a specific academic environment.

At the very beginning of its rule, Austro-Hungary set about modernising Bosnia and Herzegovina (BIH) in the spheres of public and cultural life. Industrialization began, the

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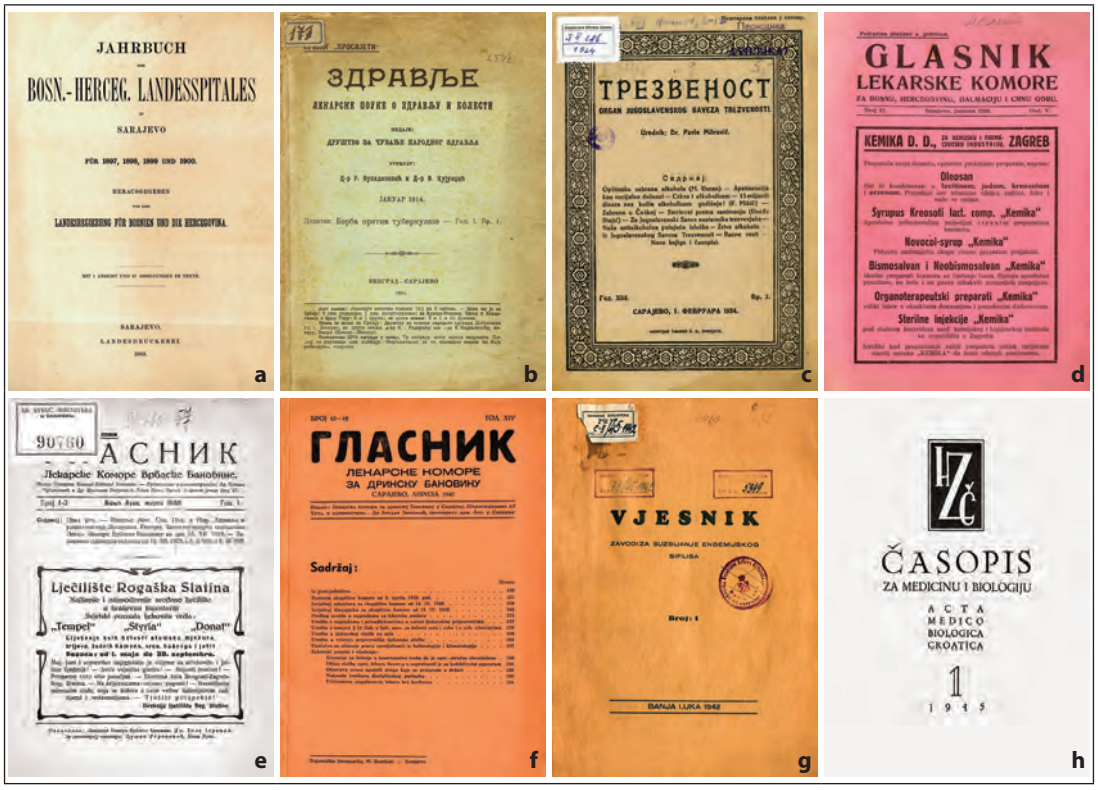
schools were reformed, the health services were organized, the first scientific research began (3), and the question of the foundation of an archaeological society and museum was raised (4), which all together created the conditions for launching different scientific journals in BIH. From then, until the end of 1945, eight medical journals were appeared in BIH (Picture 1).

This paper presents medical journals published in BIH from 1878 to 1945.

Medical Journals

Jarhbuch des bosnisch-hercegowinischen Landesspittales in Sarajevo (Yearbook of the National Hospital of Bosnia and Herzegovina in Sarajevo (Yearbook) (Picture 1a) was written in German. The first issue of the Yearbook was printed in 1894, which was also the year when the National Hospital was opened and began work in Sarajevo. A special part of the Yearbook was divided up according to various medical specializations, that is, according to the titles of the wards of the National Hospital. The chapter: "*Bericht und Arbeiten der Internen Abtheilung*" (Internal Medical Ward) was edited by Primarius Dr. Geza Kobler; the chapter "*Bericht und Arbeiten Chirurgischen Abtheilung*" (Surgical Ward) by Primarius Dr. Josef Preindlsberger; the chapter "*Beri-*



Picture 1. The title pages of medical journals in Bosnia and Herzegovina from 1878 to 1945: a) Godišnjak Zemaljske bolnice u Sarajevu (Yearbook of the National Hospital of Bosnia and Herzegovina in Sarajevo); b) Trezvenost – Organ Jugoslavenskog Saveza Trezvenosti (Temperance – the Journal of the Yugoslav Temperance Society); c) Zdravlje – Lekarske pouke o zdravlju i bolesti (Health, Medical lessons on Health and Disease); d) Glasnik lekarske komore za Bosnu, Hercegovinu, Dalmaciju i Crnu Goru (The Journal of the Chamber of Physicians of Bosnia, Herzegovina, Dalmatia and Montenegro); e) Glasnik Lekarske komore Vrbaske Banovine (Journal of the Chamber of Physicians of Vrbaska banovina; f) Glasnik Lekarske komore Drinske banovine (Journal of the Chamber of Physicians of Drinska banovina); g) Vjesnik Zavoda za suzbijanje endemijskog sifilisa u Bosni i Hercegovini (Journal of the Institute for Combatting Endemic Syphilis in Bosnia and Herzegovina); h) Časopisa za medicinu i biologiju – Acta medico-biologica Croatica (Journal for Medicine and Biology).

Picture 2. Pathological changes shown in a drawing in the Yearbook of the National Hospital in Sarajevo.

cht und Arbeiten der Abtheilung für Syphilis und Hautkranke" (Dermatology Ward) by Primarius D. Leopold Glück; the chapter: "*Bericht und Arbeiten der Abtheilung für Geburtshilflich und Gynäkolgie*" (Obstetrics and Gynaecology Ward) by Primarius and Docent Dr. Otto v. Weiss; the chapter "*Bericht der Prosectur*"(Dissection) by Prosector Dr. Roman Wodynski; and the chapter "*Bericht der Apotheke*" (Pharmacy) by Pharmacist Maks Teich (5). Physicians at the National Hospital in Sarajevo wrote a variety of medical articles in which they presented current hospital pathologies. According to Prof. Tatjana Praštalo, Doctor of Science in the field of librarianship, and for many years an employee of the National and University Library in Sarajevo, this special part of the Yearbook can be considered to be a forerunner of the first medical journal in BIH (6). The articles published were written according to the methodology of the time, and in that sense they did not lag far behind articles published in European medical journals of that time. It is interesting to note that the illustrations presenting various pathological states were in the form of drawings (Picture 2). The Yearbook was available to employees of the National Hospital in Sarajevo, but also to doctors working in smaller towns in the interior of BIH, and the wider interested public.

Zdravlje – Lekarske pouke o zdravlju i bolesti (Health – Medical Lessons on Health and Disease) (Picture1b) was a journal that appeared in BIH in January 1914 (7). On the title page, the place of publication is given as "Belgrade-Sarajevo" which would indicate that it was a journal published jointly in Serbia and BIH. However, the publisher is listed as "The Society for Preservation of Public Health" from Belgrade. The editors of the journal were Dr. Radivoje Vukadinović and Dr. Vojislav Kujundžić, also from Belgrade. The year of publication is given as 1914. In the heading on the second page of the journal it states that this was the ninth year of publication, that the journal came out in print on the 1st January, and this was no. 1. That issue of the journal, apart from the place of issue, does not contain anything that to show that it was a medical journal from BIH, which in that period had been annexed by the Austro-Hungarian Empire, and was a foreign country for Serbia. Why Sarajevo is listed together with Belgrade as the place of publication of this journal in that issue, and what the motives were for this, is still unknown and remains the subject of further speculation and research. The content of the journal comprises ten scholarly articles of an educational and informative nature, by authors from Serbia, mainly from Belgrade, written with the aim of educating the public about health.

Trezvenost – *Organ Jugoslavenskog Saveza Trezvenosti* (The Journal of the Yugoslav Temperance Society) (Picture 2c) with the parallel title in French *"Trezvenost" (Sobriete) Organe de la Ligue antialcoolique Yougslave*" from Belgrade was transferred to Sarajevo by a decision of the Committee of the Yu-

goslav Temperance Society of 3 November 1923 (8). It was published in Sarajevo from 1924 to 1926, after which it returned to Belgrade. Before it was published in Sarajevo, the Journal had already been published for 12 years, so the first year of its publication in Sarajevo was marked as Year XIII. At the time it was being published in Sarajevo, the Journal came out regularly once a month, sometimes in a double or triple edition. It was written alternately in Latin and Cyrillic script, and was distributed through its offices in Sarajevo, Belgrade, Zagreb and Ljubljana, to all corners of Yugoslavia. At that time the editor in chief was Dr. Pavle Mitrović, professor. The content of the journal comprised various scholarly articles of an educational and informative nature, in the fight against alcoholism, and articles relating to the work of the Yugoslav Temperance Society, articles about the fight against alcoholism in this country and abroad, reports from Congresses, and presentations of new national and foreign books on the harmfulness of alcohol and how to combat it, and quite often it also published advertisements by producers of non-alcoholic drinks.

Glasnik Lekarske komore za Bosnu, Hercegovinu, Dalmaciju i Crnu Goru (Journal of the Chamber of Physicians of Bosnia, Herzegovina, Dalmatia and Montenegro) (Picture 1d) was published in Sarajevo from 1925 to 1930. The editor of the journal was the respected Sarajevo physician of that time, Dr. Vjekoslav Kušan, the secretary of the Chamber of Physicians. The first issue of the journal was published on 10th July 1925, and the last in January 1930. It was written alternately in the Cyrillic and Latin script. It was planned for the journal to come out four times a year, but it was issued occasionally, when necessary, and when sufficient material had been collected. A total of 19 issues were published. They were mainly articles relating to the work of the Chamber, but also articles of a professional and informative nature, of importance for the work of doctors (9, 10, 11).

Glasnik Lekarske komore Vrbaske banovine (Journal of the Chamber of Physicians of Vrbaska banovina) (Picture 1e) was published in Banja Luka. The first issue came out in March 1930, as a triple edition (12). It was written alternately in Latin and Cyrillic script. Most probably this was also the only edition of this journal, because we have no knowledge of it being published later. The editors of the journal were Dr. Branko Čubrilović and Dr. Vukašin Babunović from Banja Luka. The content of the journal comprised articles on the foundation of the Chamber, some advertisements for various medical activities and products, and telegrams addressed to His Majesty King Aleksander I, King of Yugoslavia, and the Ministry of Social Policy and Public Health.

Glasnik Lekarske komore Drinske banovine (Journal of the Chamber of Physicians of Drinska banovina) (Picture 1f) came out occasionally from 1930 to 1941 in Sarajevo. The editor of the journal was the respected Sarajevo physician, Dr. Bogdan Zimonjić. The journal was written alternately in Cyrillic and Latin script. This was a continuation of the former *Glasnik Lekarske komore za Bosnu i Hercegovinu, Dalmaciju i Crnu Goru* (see above).

Vjesnik Zavoda za suzbijanje endemijskog sifilisa u Bosni i Hercegovini (Journal of the Institute for Combating Endemic Syphilis in Bosnia and Herzegovina) (Picture 1g) began to be published in 1942. The publisher was the institute for Combating Endemic Syphilis in Bosnia and Herzegovina (the Institute), based in Banja Luka, whose immediate work went on through the hygiene institutes of that time, public health centres and other health institutions in BIH, and sometimes in improvised in-field clinics in remote Bosnian villages. I was unable to determine how this journal came to be launched. In the foreword of the first issue,

written by Dr. Stanko Sielski, that information is not given, nor who the editor of the journal was. According to a statement made by Prim. Dr. Teodor Grüner (1913-2016), once a physician at the Institute, in March 2015, to the author of this article, the editor was Dr. Stanko Sielski. However, according to Jevtović (13), the editor in chief of the journal was Mery Atias-Günsberger, but I have been unable to establish that she was employed by the Institute. In the last issue of the journal in 1942, which came out as a double issue, in the article "Statistical Appendix II - a Brief Presentation of the First Year of Work to Combat Endemic Syphilis (14) written by Dr. E. R., it states that the Institute occasionally published a *Vjesnik* (Journal*)* in which doctors from the Institute presented their observations from the field, and discussed various epidemiological, clinical and serological questions regarding endemic syphilis, as well as questions about public health and national life, but he is not listed even in that text as the editor. The authors of the published articles were doctors at the Institute who had to sign their articles using their initials. In 1942 five volumes were issued, in that the fifth volume was marked as a double issue, 5 and 6. At the end of each issue the contents are given, and in the double issue 5 and 6 there is an appendix on 4 pages of double sided paper, with the content of the Journal of the Institute for Combating Endemic Syphilis, 1942. A total of 35 articles were printed. The journal was printed on a hectograph, and each issue had separate page numbers. The covers of the journal were printed on stiff paper in different colours. I do not know if the journal came out later, since I was unable to find it in the archives of Dr. Stanko Sielski, or in libraries in BIH, Croatia and Serbia. Drago Njegovan in his article *Jedna bibliografija periodik Nezavisne Države Hrvatske* (1941-1945) (A Bibliography of Journals of the Independent State of Croatia, 1941-1945), (15) under number 237, mentions the publication "*Vestnik Zavoda za suzbijanje endemijskog sifilisa*" *(Journal of the Institute for Combating Endemic Syphilis)*, Banja Luka, II/1943. Biblioteka Zbora liječnika, Zagreb — 5652. When I traced this information I did not find that issue of the journal. However, it is strange that this bibliography did not mention any issues of the journal from 1942. After the first three issues of *Vjestnik zavoda za suzbijanje endemijskog sifilisa*, A.V. (A.V. correctly decoded: Dr. Ante Vuletić), at that time the editor in chief of *Liječnički vjesnik (Medical Journal)*, and one of the organizers of the campaign to combat endemic syphilis, as well as to save his Jewish colleagues, published a presentation of those three issues of the journal, in which he states: "With the participation of 125 experts, 78 doctors and their observations it is completely understandable that such a major campaign does not have a purely practical purpose, that is, to wipe out once and for all endemic syphilis as an endemic disease, but also has the task of studying and to clarify many issues related to endemic syphilis. So in this way the campaign from a scientific point of view is making a valuable contribution to international venereology." In the text that follows he is critical of the linguistic roughness of the articles, but also recognises the head of the Institute, Dr. Stanko Sielski, who in those difficult circumstances still managed to launch a scholarly journal (16).

Časopis za medicinu i biologiju (Journal for Medicine and Biology) with the parallel title in Latin Acta medico-biologica Croatica (Picture 1h) appeared in BIH at the beginning of 1945. In the *Impressum* on the back page, under the title of the journal, it says: "founded by the medical faculties to mark the opening of the Medical Faculty in Sarajevo". The publisher of the journal was the Hrvatski izdavalački bibliografski zavod (The Croatian Publishing and Bibliographic Institute) of Zagreb. The chairman of the ed-

itorial board was Prof. Dr. Ante Šercer, Dean of the Medical Faculty in Zagreb, and the editor of the journal was Dr. Josip Körbler, a professor at the Medical Faculty in Zagreb. The secretaries to the editor were Vjekoslav Duančić and Stanko Sielski, the Dean of the newly opened Medical Faculty in Sarajevo at that time. It was planned for the journal to come out four times a year. The first issue of the journal had eight articles written by authors from Zagreb. Each article had an Abstract in German, and a list of the literature used. A presentation of a book, *Unutarnje bolesti* (*Botteri-Budak*) (Internal Diseases) was also published. The journal also had these sections: Reviews, Presentations and News. It is interesting to note that this journal, after the Second World War, that is, after the break-up of the so-called Independent State of Croatia, was not issued either in Croatia or BIH, and the Medical Faculty in Sarajevo, for whose foundation in 1944 the journal was launched, was closed.

Medical Articles in Non-Medical Journals

Articles on medical subjects in the period from 1878 to 1945 were quite often published in journals issued by cultural and educational institutions, and cultural and educational societies. Some of those non-medical journals had special sections in which articles were published in the field of medicine, or several medical articles were published in a single issue.

One of those journals was *Glasnik Zemaljskog muzeja u Bosni i Hercegovini* (The Herald of the National Museum of Bosnia and Herzegovina) (Herald), which began to the published in 1889 and was the first journal to be published in BIH with scholarly and scientific ambitions. As a multi-discipline and inter-discipline journal (17) it often published articles from the field of medical ethnography and anthropology. The journal was published in the Bosnian language, and it was written alternately in Cyrillic and Latin script. Up to the end of Austro-Hungarian rule in BIH, the Herald) had published 35 articles whose contents related to public medicine and medical anthropology. The articles are interesting for the medical profession in BIH for many reasons and as such deserve to be the subject of new research, especially when we know that some of them were translated into German and published in the publication *Wissenschaftliche Mitteilungen aus Bosnien und der Hercegowina* (18, 19). Other non-medical journals occasionally published articles on medical topics. Examples of this are: Školski vijesnik, (School Journal) a journal published by the national government for Bosnia and Herzegovina published an article in 1905 "How Bosnian teachers can make a major contribution to the improvement of health" written by Dr. Justin Karlinski (20), the journal *Prosvjeta*, (Education) the Journal of the *Prosvjeta* society (Education), even had a separate section in issue 9 in 1919 entitled "Public Health" with 18 articles from the field of medicine, written on pages 149 to 180 (21), *Novi Behar,* (New Be-Har) the journal of the Muslim cultural society "*Narodna uzdanica*", (National Hope) in issue 12-13 of 1944 published four medical articles (22) and *Napredak,* (Progress) the Croatian national calendar, the journal of the Croatian cultural society of the same name, based in Sarajevo, in its second issue in 1908, published a medical article entitled "On Phthisis and Its Treatment (using juniper leaves)" (23).

Medical articles published in non-medical journals were mainly educational in nature, aimed at educating the general public regarding health matters. They were mainly written by doctors, but they were written in everyday language, in a simple style to make them accessible to a wide circle of readers. As far as I know, these articles have not yet been the subject of research, nor has any-

thing been written about them, so that they are interesting for future research.

Conclusion

The medical journals mentioned above were issued or appeared in BIH at times when it was not independent, and was under the influence of the health culture of the occupying authorities and ruling regimes, at times which were marked by specific political and social circumstances. Most of the authors whose articles were published in these journals were citizens of the occupying authorities, although the articles published were mainly the result of research undertaken in BIH. Of all these journals it may be said that they were published for a short time and that they mainly contained scholarly medical journals, which dealt with the health care and medical situation in BIH from various points of view. Medical articles published in non-medical journals in the period from 1878 to 1945 have not yet been the subject of research, nor has anything been written about them, so that they are interesting for future research.

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Authors' contributions: Conception and design: HT; Acquisition, analysis and interpretation of data: HT and ET; Drafting the article: HT and ET; Revising it critically for important intellectual content HT and, ET; Approved final version of the manuscript: HT and ET.

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Historical article

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The English Sweating Sickness: Out of Sight, Out of Mind?

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In this paper we aim to add additional knowledge regarding the occurrence, origin and epidemiological features of the English sweating sickness. The English sweating sickness raged in five devastating epidemics with mortality rates between 30 and 50% between 1485 and 1551 throughout England, and on one occasion also affected mainland Europe, in 1529. The Picardy sweat, generally considered as the English sweating sickness' lesser deadly successor, flared up in France in 1718 and caused 196 localized outbreaks with varying severity all over France and neighboring countries up to 1861. The English sweating sickness has been the subject of numerous attempts to define its origin, but so far all efforts have failed due to lack of material, DNA or RNA, that - using modern techniques and knowledge - could shed light on its cause. Although the time frame in which the English sweating sickness occurred and the geographical spread of the outbreaks is generally known, we will demonstrate here that there was more to it than meets the eye. We found reports of cases of sweating sickness in years before, after and between the 1485, 1508, 1517, 1529 and 1551 epidemics, as well as reports of sweating sickness in Italy and Spain. **Conclusion.** In spite of the fact that the English sweating sickness apparently has not caused casualties for a more than a century now, we suggest that -given the right circumstances- the possibility of re-emergence might still exist. The fact that up until today we have no indication concerning the causal pathogen of the English sweating sickness is certainly not re-assuring.

Introduction

The English sweating sickness (hereinafter referred to as ESS) struck, according to general belief, for the first time at the very beginning of the reign of Henry VII, in August 1485, and re-emerged in 1507, 1517, 1528 and 1551. Webster however mentioned that –according to Erasmus- the ESS first appeared in 1483 and re-emerged in 1485 (1), while, an account in the York Civic Records mentioned a pestilence in the area (N-E England) in June 1485, that bore a great resemblance to the sweating sickness. This was three months before the Battle of Bosworth Field that brought an end to the War of the Roses and the reign of Richard III (2). The irregular intervals between the five epidemics (22, 10, 11 and 23 years respectively) suggest an endogenous, ecological or meteorological trigger.

The fact that the onset of the disease was quick, deadly and without warning invariably caused panic in the affected population. Holinshed wrote: "*so sharp and deadly that*

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the lyke was never hearde of to any manne's remembrance before that tyme" (3). The ESS was new and unknown, struck without warning, killed often hundreds per week and disappeared apparently for no reason after one or two months. These short episodes did not however cause a general population decline, as did the plague in the 14th century (4). The first symptoms were chills and tremors, quickly followed by high fever and great weakness. The body was covered with perspiration but a rash was rarely reported. The course of the disease was exceptionally violent and sometimes fatal within hours. The mortality rate was highly variable, but probably between 30 and 50%. In comparison, the overall mortality from the 1346-1353 plague epidemic was estimated at 30% to 60% (5, 6). Zinsser cites ESS mortality as high as 80 to 90% (7), but Creighton reports a mortality of 5% (8). The ESS thus emerged around 1480, caused five major epidemics [1485, 1507, 1517, 1528, 1551], and then apparently disappeared, until a more benign variant -the Picardy Sweat- emerged in France more than 150 years later (9).

In the 14th century, the Black Death (*Yersinia pestis*) caused the worst natural disaster that mankind has ever experienced, and considerably reduced the population in both Asia and Europe (10). Both continents recovered slowly and despite the fact that political and religious turmoil hampered progress, the fifteenth and sixteenth centuries produced significant progress in science, medicine and in general in the quality of life. Besides the plague, several other new diseases emerged in this era, e.g. syphilis, the Dancing Mania, influenza and the ESS or Sudor Anglicus. Some were here to stay; others apparently disappeared without a trace (i.e. the Dancing Mania (11) and ESS). Europe however did not have the monopoly on devastating epidemics. A new disease called Huey Cocoliztli, emerged in Mexico for the first time in 1545-1548, and reemerged in 1576-1578. These two epidemics killed an estimated 5–15 million and 2-2.5 million people, respectively, or up to 80% of the native population. It was remarkable that the disease affected only the local Aztec populations and left the Spanish invaders untouched, like the ESS that apparently struck the English by preference. Acuna-Soto and co-workers give a summary of symptoms that vaguely resemble those of what we call ESS: "*The disease had a very short course, lasting three to five days. It started abruptly with high fever, vertigo, severe headache, insatiable thirst, red eyes and weak pulse. Patients became intensely jaundiced, very anxious, demented and restless. They did not tolerate any blanket over their skin…*." (12-14). It was also suggested that the Huey Cocoliztli was a viral hemorrhagic fever with a murine vector (15, 16).

As the plague, influenza appears to be an ancient disease. The symptoms of human influenza were already described by Hippocrates, roughly 2,400 years ago (17). The virus seems to have caused epidemics throughout human history (18). With the arrival of Christopher Columbus, the virus was introduced to the Americas, and almost the entire indigenous population of the Antilles was killed by an epidemic resembling influenza that broke out there in 1493 (19). The best known and probably the most lethal outbreak was the so-called Spanish flu pandemic (type A influenza, subtype H1N1) which continued through both 1918 and 1919, with an estimated death toll of 50 to 100 million people worldwide (20).

In this paper we aim to add additional knowledge regarding the occurrence, origin and epidemiological features of the English sweating sickness.

Out of sight, out of mind

The history of the world is intertwined with that of infectious diseases. The impact, in

the absence of effective medication, on the population is in some cases devastating, especially when the cause is unknown, and superstition (meteorites, earthquakes, divine punishment, etc. were often implicated) creates confusion and fear. Nevertheless, efforts to explain disease and infection more scientifically have been made throughout history, Hippocrates wrote about the spread of disease by means of air, water and location, and constructed hypotheses on the association between diet, climate and living conditions (21). In contrast to our era, in which we address epidemics with concerted and international action, epidemics (plague, syphilis, smallpox, cholera, yellow fever, typhoid fever, and other infectious diseases) occurred in antiquity and in the pre-20th century era regularly and were in a sense the norm (22). In most cases there was no effective treatment available. A hallmark of various epidemics was that they were a constantly lurking danger. A few, e.g. ESS, Dancing Mania, and Huey Cocoliztli, emerged, raged for years or even decennia, and apparently disappeared without good reason.

From what we know today about infectious diseases, i.e. they are caused in general by bacteria and viruses, it is probable that a certain infectious disease will not spontaneously disappear unless we eradicate the causal agent, vaccinate or medicate. In other words: very few pathogens vanish without a trace. In 1992, Lederberg and co-workers defined six categories of factors that could explain the emergence or re-emergence of infectious diseases. These factors are: human demographics and behavior; technology and industry; economic development and land use; international travel and commerce; microbial adaptation and change; and the breakdown of public health measures (23).

From what we know about the fifteenth and sixteenth centuries (see also the next chapter), five of the six determining factors played their role, there is only no evidence of microbial adaption. Why then did ESS apparently disappear after 1551? Perhaps the medieval ESS epidemics were the proverbial tip of the iceberg, an accepted characteristic of most zoonotic disease manifestations. The causal agent might still be around, but the triggering factors for emergence of ESS are now no longer present. There is, however, no guarantee that, should Lederberg's six factors apply again, re-emergence is out of the question.

Setting the scene: England during the peak of the English sweating sickness (fifteenth and sixteenth centuries).

Central to all social change in the fifteenth century was the occupational shift. As a result of the human toll the plague took in England in the 14th century, villein (serf) labour largely disappeared. Landlords increasingly abandoned direct management of their estates in favour of a leasehold system. In many cases they faced growing arrears of rent and found it difficult to maintain their income levels. They solved the labour shortage problem by converting their holdings to sheep pasture but, in order to achieve this, massive land enclosure took place. This meant deforestation on a massive scale and the subsequent important disruption of fauna and flora. This period could also be considered as the golden age for the English labourer, although individual prosperity varied widely. There was a well-developed land market system among peasants, some of whom managed to rise above their neighbours and began to constitute a yeomen class that extended their trade beyond the island, trading with, for instance, France, The Netherlands and Germany, which became important trading partners.

Remarkable and peculiar is that, although the overall living standards improved, sanitary conditions in English medieval cities,

streets and houses had a very bad name all over Europe. This was mentioned by several contemporary authors. Jortin mentions: "*Erasmus, speaking of the sweating sickness, in a letter to Franciscus, Wolsey's physician, ascribes the sweating sickness, partly to the incommodious form and bad exposition of the houses, to the filthiness of the streets, and to the sluttishness within doors. The floors, says he, are commonly of clay, strewed with rushes, under which lies unmolested an ancient collection of beer, grease, fragments, bones, spittle and excrements of dogs and cats, and everything that is nasty…*" (24). Ironically, London in fact needed the Great Fire of the 2nd to 6th September 1666 to more or less clean up the city. Despite their vital importance for trade and commerce, towns remained as a rule small, with London as the exception. From a population of about 60,000 or 70,000 around 1500, it grew to about 250,000 inhabitants by 1600, and this exponential growth probably made it a prime target for epidemics.

The identity and origin of the English sweating sickness

This suggests that a combination of environmental disruption, more intensive interhuman contact through trade and a growing population facilitated the largely unnoticed transmission of the disease in rural areas, up to the point when the pathogen hit the cities and found ample opportunity in the poor sanitary conditions to cause epidemics that caught attention because of their magnitude, and because ESS also affected and even seemed to preferentially target the rich and mighty. It gained a fearsome reputation.

An extensive search for records of ESS cases (more than 800 books, manuscripts, scientific papers, reports, journals and city archives dating from the 16th to 21th centuries from English, French, Dutch, German, Italian and Spanish authors, and Turkish and Russian literature translated into English, French or German), showed that before, after and between the five major epidemics, sporadic cases occurred in Britain, as well as in mainland Europe. This suggests a source, most likely animal, that was always present, although probably in fluctuating numbers, but only triggering major epidemics when the population peaked.

In a previous publication, we have already described in detail what we assume to have been the most likely candidate for ESS, i.e. a hantavirus, probably now extinct or mutated (9). A candidate also suggested as the cause of the ESS was an ancient variant of anthrax. However, in the fifteenth and sixteenth centuries, Britain was divided in two major agricultural regions. South of the "watershed" line that roughly runs from Liverpool to Grimsby, the main agricultural activity consisted of grain production, while north of the line the production of livestock, mainly sheep but also cattle, prevailed. It was estimated that in the 15th century there were five times more farm animals (cows and sheep) than humans in that northern region. Should anthrax (the cause of "woolworkers disease") have been the cause of ESS, it would appear logical that the North rather than the South would have been affected. In the southern part of Britain a rodent-borne disease, such as a hantavirus disease, was however more likely to occur than in the North because of the specific food available (25). It has been well established that the ESS only struck England and Wales, never Scotland. Despite an intensive search on our side, we were not able to retrieve reports or even short notes on any increased presence of rodents/ticks/lice/mosquitoes in the ESS epidemic years. Animal-to-human transmission or human-to-human transmission was clearly not an option for contemporary scientists. The absence of such reports makes it difficult to find an explanation for the emergence and re-emergence

of the ESS. On the other hand, we noticed solitary ESS cases in many years in-between the five epidemics, which would suggest the constant- presence of the causal agent and vector, but much less in non-epidemic years. It should also be taken into account that in the 15th-16th centuries, England (which had a bad reputation for domestic cleanliness all over Europe) that all sorts of animal species could frequent habitations on a regular basis.

It is remarkable that the emergence of ESS coincided with the Ottoman invasion in Eastern Europe. There is however no convincing proof for the often cited suggestion that the sweating sickness originated from the Ottoman attack on Rhodes (1480) and was brought to Western Europe by soldiers returning from that war in 1483. Also there are no reports of any ESS-like epidemic amongst Ottoman troops at Rhodes or anywhere in Eastern Europe during their conquest (1453-1683). Bordier writes: "....*In the south it* (the sweating sickness) *invaded Württemberg, the Duchy of Baden, the Palatinate, Bavaria, and Vienna, which at that time was being besieged by the Turks. This was its extent and it did not strike the Turks. …*" (26). It thus appears that is was, and often still is, easier to blame " foreigners" without good reason or proof, than to look for the reason closer to home. In the case of the ESS, the "foreigners" were the Ottoman Turks or the "French" mercenaries (in reality many nationalities, i.e. Swiss and Germans, were represented in that group) hired to fight the Battle of Bosworth for Henry VII.

It thus also seems highly unlikely that the ESS was imported from France by Henry's mercenaries (an unsupported assumption by John Caius, written down more than 60 years after the facts) as the ESS was never mentioned in France around 1485. There is also no mention of ESS victims in Henry's army during the landing in Cornwall on the 7th August or during the Battle of Bosworth (22nd August 1485). A few days after the Battle of Bosworth, the ESS broke out in Oxford, a city more than 120 km away from Henry's allegedly infected army (27). It seems highly unlikely that the disease could have travelled from Henry's army to Oxford in such a short time.

There is, on the other hand, evidence that the ESS raged in York (180 km North of Bosworth) in 1485 (28) and it could be that various local troops that were joining the army of Richard III (Richmond's adversary and then king of England) were infected during their march to the South and transmitted the disease during and after the Battle of Bosworth to the troops of Richmond (later Henry VII). This hypothesis allows two possible carriers for the disease to London: the victorious Richmond army and parts of the defeated army. The disease could thus well have been local rather than foreign. At the same time, this hypothesis would argue for human-to-human transmission. We have already examined the possibility of human-to-human transmission in a previous paper (9) and found that the epidemic progress fitted 16th century travelling times, i.e. travel on foot, with luggage: 15–20 km/day (75-100 km per week), travel on horseback, no spare horse: 30-40 km (150-200 km per week), travel on horseback, with a spare horse: 40-60 km (200-300 km per week). According to von Marval writing about the progress of ESS in Germany in 1529, these travel speeds are in alignment with the epidemic's progress. Although it should be said that the wise traveler would prefer travelling by water (sea or river) as this was much safer, less exhausting and even faster most of the time, when a detour had to be taken. The same author -Southwood, (28) cited Holinshed who mentioned that in 1252 "*sweats, agues and other diseases…*" prevailed in England (29). Holinshed unfortunately did not specify further.

Dr Dyer's research, based on parish registers, represents a comprehensive analysis

of the demographic impact of the five outbreaks of the sweating sickness. Contemporary impressions of the strong age, class, and sex characteristics of the victims of the sweating sickness (young, rich males) are modified to give a more dispassionate and informed picture. He also shows how the sweating sickness was predominantly a rural rather than an urban disease, with a limited overall demographic impact, and that there may have been occurrences outside the five "classic" epidemic years (30). The underlying hypothesis is that the causative agent of the sweating sickness was spread by human-to-human contact as well as initially through a zoonotic or an environmental vector. Dyer shows that, when London was affected in the early weeks of July, there was a distinct male preponderance of victims, although the total number of deaths was not strikingly high. This male preponderance was not always paralleled outside the capital.

The bummock of the iceberg

In zoonotic diseases, the "tip of the iceberg"[1] principle, i.e. the fact that the outbreaks or epidemics we observe in epidemiological surveillance only represent the obvious part of the total outbreak, is well known. The "bummock of the iceberg" is that almost invisible part that often escapes the attention and reporting because solitary cases occur.

It is widely accepted that ESS indeed caused five important epidemics and had a major impact in the affected regions. In addition, our literature search revealed several other records of ESS victims, often outside the timeframe of the five epidemics (Table 1). The ESS apparently appeared, according to the records, for the first time in 1483 (1) (32) and was last described in Röttingen, Germany as late as 1802 (33). The time span between the first and last documented appearances thus was a staggering 309 years (1493 to 1802), which indicates that is was not at all a one-day fly. We have found 20 different references so far accounting for one or multiple consecutive years in which the ESS took its toll (Table 1).

All the epidemics affected England, but in 1529 most of Europe suffered heavily from ESS. From England the disease passed on the 24th July to Hamburg, Germany, by ship (34), turned West towards The Netherlands and Flanders, where it caused casualties in September. Given the intensive trade by ship between England and The Netherlands, is remarkable that the ESS apparently arrived overland rather than by sea.

In late September 1529 it reached Denmark and the Scandinavian Peninsula. In Sweden, Magnus Eriksson, the son of King Gustav Vasa succumbed to it (34, 35). No precise records could be found regarding the appearance of ESS in Russia (36) (or Muscovy, as the Moscow duchy was called up until 1480). Even Hecker was not sure when he reported: "*That the Sweating Sickness likewise penetrated into Lithuania, Poland, and Livonia, if not into a part of Russia, we know only in a general way*" (37). Although concise, we also found a reference to the occurrence of the ESS in 1529 in Cremona, Northern Italy (38, 39). This was perhaps not all too surprising as in that year most of Europe suffered from the disease.

There are also some rare references to ESS outbreaks in Spain, unfortunately without mention of dates or locations. Von Niemeyer wrote: "*Regarding the geographical range of sudor anglicus….. Its home is in France, southwest Germany, and Italy, while in the Netherlands, middle and northern Germany, and latterly in Spain, it has only been met with in occasional epidemics*" (40). Heylyn noted that, as also seen in Calais, France in 1517, only the English fell victim to the ESS

[1] For those of us that are not encountering icebergs on a daily basis: the hummock is the part of an iceberg above water, the bummock is the part under water (31).

Table 1. Occurrence of the English Sweating Sickness over time

	Year	Country	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	OCT	NOV	DEC
1	1483	England	First appearance of the English Sweating Sickness according to Erasmus [1]											
2	1485	England	-							Bosworth [2]	London (15000)			
3	1489					Netherlands, Belgium, continued for 2 yrs [3]								
4	1490													
5	1491-1492	Ireland						Cork [4], Meath [5]						
6	1508	England								London, Greenwich, Eltham, Chester(91) [2]				
7	1511	England								Cambridge [2]				
8	1517	England								London(10000), Oxford(400), Cambridge, Chester [2]				
		France								Calais				
		Germany								[6]				
9	1522							Norfolk, England - [7]						
10	1526	Flanders						Ghent (33) [8]						
11	1528	England						London (2000), Kent [2]						
		France							Calais [2]					
12	1529	England												
		Ireland												
		Italy	[9]											
		Flanders									Antwerp (500), Bruges (300), Ghendt (300), 15/09 Liège [10]	Malines		
		France						Aix (1000) [11]						
		Netherlands								E-Friesland	Amsterdam	03/10 Zierikzee		
		Germany							Hamburg 25/07 (2000), 31/07 Lübeck(), Bremen(), Verden(),	14/08 Mecklenburg, 27/08 Stettin, Wismar, Demmin, rostock, Stralsund, Greifswald, Hanover, Göttingen, Brunswick, Lüneburg, Waldeck, Einbeck, Hadeln,	01/09 Danzig, 08/09 Königsberg, Westphalen, Weser valley, 11/09 Frankfurt, 13/09 Worms, 30/09 Marburg, 15/09 Julich, Cologne, 24/09 Speyer	06/10 Augsburg, 24/10 Strasbourg, Freiburg, Mühlhausen, Gebweiler	Würtenberg, Baden, Upper Rhin, Constance Lake,	

Continuation of Table 1. Occurrence of the English Sweating Sickness over time

	Year	Country	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	OCT	NOV	DEC
12	1529	Austria								Vienna 22/9-14/10	[12]			
		Denmark, Scandinavia								29/9 [13]				
		N-E-Europe							Livonia, Lituania, Poland, Russia, Copenhagen, [14]	Prussia, (Thorn, Kulm) (30000) [14]			Bern [15]	
13	1530	C-Europe	Basle [16]											
14	1529-1534	France								Trousse Galant [6]				
15	1543	Ireland						Galway [17]						
16	1551	England			22/03 Schrewsbury (900)			Loughborough (19), Oxford(160)	London (872)	Devonshire(27) [2]				
		Netherlands												
		France						Trousse Galant [18]						
17	1578-1579							Colchester [2]						
18	1592	Netherlands						Kleef [19]						
19	1644	Cornwall									[20]			
20	1802	Germany								Röttingen [21]				

In yellow: month(s) of occurrence of English Sweating Sickness, if reported. Exact dates if reported; ()=The number of reported deaths (probably estimates and, most likely; underestimated numbers), if reported; []= reference.

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in Spain: "*The sweating sickness…., although this should be in an entirely different climate, as in the present instance was the case with the English who were living in Spain …*" (41).

Intriguing reports on the sweating sickness appear in Bryden's "Epidemic Cholera in the Bengal Presidency." (42), Murray's "On the Malwah Sweating Sickness." (43) and Orton's "An Essay on the Epidemic Cholera of India." (44). The ESS is said to have appeared in Malwa (1839) in Central-India; Meean Meer (1852), the former Lahore Cantonment during the British period; Peshawar (1855), in North-West Pakistan; and Agra (1859), in the northern state of Uttar Pradesh, India (42). The chances are however that, given the geographical and time discrepancies, these epidemics were of another nature than true ESS, most probably severe or fatal cholera cases.

An intriguing question is why there were such irregular time gaps between outbreaks. The ESS was typically a summer phenomenon, but did not occur every summer, and the pattern was indeed highly irregular (22, 10, 11 and 23 years between epidemics, but with cases in-between). The best guess currently is that the ESS outbreaks corresponded to fluctuations in the climate. Random periods of intense rainfall or flooding, or temperature differences which were known to trigger outbreaks over the decades, could account for the seemingly haphazard timing of the outbreaks. It is however plausible that the population densities of an animal carrier of the causal pathogen could respond to climatic changes, which in turn could explain the pattern. The reported flooding, or long and heavy rainfall preceding the outbreaks could also be responsible for driving the carrier to human habitation for cover and food. Unfortunately, we were not able to find reliable references to numbers of pest species of any kind in the late medieval European literature.

The possibility of another causal agent, besides a hantavirus, cannot be ruled out. Table 2 compares the most common clinical features of the most likely agents of ESS. In our opinion, plague, malaria (the ague, as it was called), influenza and ergotism (most likely the cause of the Dancing Mania) can be ruled out, as those infections were all too well known in those days. Although Yellow Fever, Dengue and Chikungunya could present with similar clinical signs, we fail to see how those pathogens and their vectors would have established themselves for decades in wet, cold England, and only affect the English. From our table, the most likely candidates would be a hantavirus, anthrax and CCHF. CCHF lacks some key signs, and only inhalational or gastrointestinal anthrax infection could give the mortality rates of ESS. There are, however, no contemporary reports of mass animal deaths in those days

Table 2. Clinical symptoms of the most likely candidates for English sweating sickness

Disease	English Sweating Sickness	Hantavirus infection	Omsk Hemorrhagic Fever	Bacillus anthracis	Typhus (Rickettsioses)	Crimean Congo Hemorrhagic Fever	Ergotism (ergotamine)	Ockelbo disease	Rift Valley Fever
Vector	?	Rodents, bats, shrews	Dermacentor reticulatus, D. arginatus, Ixodes persulcatus	Infected animals or contaminated animal products	Lice	Hyalomma, Rhipicephalus and Dermacentor ticks	Claviceps purpurea contaminated grains	Sindbis virus	Culex spp, Anopheles spp
Symptom	Clinical manifestations								
Chills	x	x	x	x	-	-	x	-	x
Headache	x	x	x	x	x	x	x	x	x
Myalgias	x	x	-	x	-	x	-	x	-
Arthalgias	x	x	-	-	x	x	x	-	-
Tachycardia	x	x	-	x	-	-	-	-	-
Tachypnea	x	x	x	x	-	x	-	-	-
Chest pain	x	x	-	-	-	-	-	-	-
Shortness of breath	X (severe)	x	x	x	-	x	x	-	-
Sweating	X (severe)	x	-	X (severe)	-	-	-	-	-
Nausea	-	x	x	x	-	x	x	x	x
Cough	-	-	-	-	-	x	-	-	-
Vomiting	-	x	x	x	-	x	x	-	-
Malaise	-	x	-	x	-	x	-	-	-
Abdominal pain	-	x	-	x	-	x	-	-	x
Diarrhea	-	x	-	-	x	x	-	-	-
Fever	x	x	x	x	x	x	-	x	x
Enlarged lymph nodes	-	-	-	-	x	x	-	-	x
Fatigue	-	x	-	-	-	x	x	x	-
Rash	-	-	-	-	-	x	-	x	-
Other						Bleeding			Bleeding

that could explain a nationwide (and in 1529 a continent-wide) epidemic of anthrax disease. Hantaviruses (those that are around today) lack the possible human-to-human transmission, but the ESS bears a fair resemblance to New World hantavirus infections (that are not present as such in Europe today). We hope new techniques or new findings in the contemporary literature will shed light on this subject, but we fear that with the present knowledge of the ESS, we are stuck with the bold Sherlock Holmes' citation: "*When you have excluded the impossible, whatever remains, however improbable, must be the truth.*"

The fact is that the last three decades of the 15th century and first half of the 16th century appear to have been much warmer than the previous 150 years, when the temperature steadily declined after the Medieval Warm Period (1000-1400) (45). It was apparently warm enough to bathe in the Rhine in January in the first half of the 16th century. However, the winter of 1564-65 was the first of many long and bitterly cold seasons (46).

Taking into account that reporting of infectious diseases was not systematic in the 15th and 16th centuries and accounts often appear as a single sentence in a manuscript of several hundreds of pages, it is very pos-

Picture. Epitaph in the Saint Nicolas' Church, Ghent, Belgium with the text: *Hier licht begraven Olivier van Minjau ende Amelberghe Slangen syn wettelick wyf was ende hadden te samen eenendertich kinderen met eens-maels thien dochters ende de rest zonen twelck altzamen ghestorven zijn vader moeder ende kinderen in ougsti 1526*". (Here lies buried Olivier van Minjau and Amalberghe Slangen, his wife, together with their 31 children, 10 daughters and 21 sons, who all died in August 1526). Source: Paul Heyman 2017.

sible that more ESS cases occurred than we have traced and that we also missed some manuscripts that could give further information. Some accounts may also have been lost when manuscripts were destroyed. The complete history of the ESS will probably never be written.

However concise, sometimes the information is remarkably detailed and close to home. In Saint Nicolas' Church , Ghent, Belgium, an epitaph is displayed with the text: "*Hier licht begraven Olivier van Minjau ende Amelberghe Slangen syn wettelick wyf was ende hadden te samen eenendertich kinderen met eensmaels thien dochters ende de rest zonen twelck altzamen ghestorven zijn vader moeder ende kinderen in ougsti 1526*" (Author's translation: *Here lies buried Olivier van Minjau and Amalberghe Slangen, his wife, together with their 31 children, ten daughters and 21 sons, who all died in August 1526*) (Picture). The cause of death apparently was the ESS, and they all perished within a month of each other, six months after they witnessed the Entry of Emperor Charles V into Ghent on the 24th February 1526 (47-49). This happened therefore three years before the ESS was reported in mainland Europe in 1529, and demonstrates that ESS mortality could be extremely high.

The only disease that resembles the ESS is the Picardy sweat, also called, in French, "la suette des Picards", or "Frieselfieber" in German. It first emerged in the Picardy region (N.W. France), hence its name, in 1718 and caused 196 outbreaks in total between 1718 and 1861, which were mostly localized and often, but not always, more benign than the ESS (50). The Picardy Sweat was mostly confined to France, although not only to the Picardy region, but it was also known in Germany, Belgium, Austria, Switzerland and Italy. Since it emerged some 150 years later than the ESS, much more research has been done into its nature, origin and treatment, and a clear link to rodents was established (51) but its true nature also remains unknown.

Conclusion

The English Sweating Sickness, the Picardy Sweat and the Huey Cocoliztl are three in a select group of emerging diseases that have largely escaped medical science. Hantavirus infection remains a possibility, on the basis of the resemblance of the first two to Hantavirus Cardiopulmonary Syndrome or Hemorrhagic Fever with Renal Syndrome, with or without pulmonary involvement.

The contemporary observations that the ESS was clinically different from all "plagues" known at that time, indicates an unknown candidate, possibly viral, and most likely zoonotic. The nature and origin of the English Sweating Sickness, the Picardy Sweat and the Huey Cocoliztl are still medical mysteries and will most probably remain so. We have attempted to demonstrate here that the ESS was more common than generally thought for a long period of time.

As to its origin, ESS was most likely native to England. Neither the assumptions of importation by mercenaries from France not importation from Rhodes seem probable, given the lack of evidence. We investigated the possibility of the transmission of ESS via the Ottoman route, but found nothing to sustain that hypothesis. At the time of the emergence of ESS the Ottomans were reigning in Turkey of course, but they also gradually conquered Eastern Europe (1470 to 1683) up to Vienna. Also in those regions we found no evidence of ESS-like manifestations. The finding was interesting that travel by sea from the Mediterranean Sea Basin to Western Europe in those days was problematic, due to the fact that most major harbors were occupied or besieged by the Ottomans. Therefore, the few Englishmen that fought the Ottomans had to return over land. This took months of travel and again it seems un-

likely that they could: a) carry the pathogen or vector with them for so long and b) not start an epidemic along the route.

As many so-called new viruses have emerged or re-emerged in recent decades (HFRS, HCPS, SARS, HIV, MER-coV, Zika, etc.), the English sweating sickness is, however unlikely, still a candidate for re-emergence. What is known on this topic is still too little, as there is no definitive evidence concerning the causal pathogen of ESS. From contemporary writings we can however distill a fairly accurate epidemiological picture. The fact is that ESS had a considerable impact on the European population in the 15th and 16th centuries, as did the Picardy Sweat in the 18th and 19th centuries.

What this study adds to current knowledge is further confirmation of the origin, i.e. the suggestion that it may be a hantavirus, the occurrence of ESS cases in Southern Europe (Spain, Italy), indications that the ESS could have caused casualties well before the generally accepted date of 1485, further indications that, contrary to the assumed foreign origin of ESS, the disease was indigenous to England, and proof of more cases in between epidemics.

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A Quarter Century of Emerging Infectious Diseases – Where Have We Been and Where Are We Going?

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A quarter century ago the landmark report from the U. S. National Academies of Sciences, Engineering, and Medicine entitled *"Emerging Infections, Microbial Threats to Health in the United States"* was released. This classic study captured the societal changes then underway in our rapidly growing world: The growth of the world's population and changing human behavior; the advances and globalization of technology and industry; the changes in economic development and land use; the dramatic increase in speed and frequency of international travel and commerce; the adaptation of microbes and the appearance of never before seen pathogens; and the breakdown of traditional public health measures. This societal evolution has only increased and the growing frequency of outbreaks foretold in the report has come to pass. Each new disaster has precipitated changes and adaptations in our global response to infectious diseases designed to reduce risks and avoid future outbreaks. We discuss these past events and how each led to change in an effort to mitigate future threats. We also look to the future to consider what challenges might lay ahead. **Conclusion.** Major outbreaks over the past quarter century validated the concept of emerging infectious diseases and led to improvements in global policies and national public health programs; however, there will likely always be new diseases and the threat of reemergence of diseases once thought controlled leading to a constant need for vigilance in public health preparedness.

Introduction

The 1992 publication by the United States National Academies of Sciences, Engineering, and Medicine (NASEM) entitled *"Emerging Infections: Microbial Threats to Health in the United States"* was a watershed event that captured the many societal changes taking place around the world that impacted human health and resulted in the christening of a new term, "emerging infectious diseases" which continues to be relevant even now, a quarter century later (1). The speed of international travel and commerce coupled with advances in technology, increases in the world's population, changes in human behavior, expansion of communities into areas previously uninhabited thereby bringing people into close contact with zoonotic pathogens, and a general breakdown in public health had all combined to set the stage for recognition of diseases hitherto unknown and the emergence of resistant microbes resulting from microbial adaptations. The discovery of HIV/

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Table 1. Selected outbreaks and technological advances of international importance that validate the concepts of Emerging Infections, 1992 to the present

Notable Outbreaks, 1992-1995
Hantavirus in the Americas, 1993
Plague in Surat, India, 1994
Hendra virus in Australia, 1994
Ebola Hemorrhagic Fever, Kikwit, DRC 1995
Notable Outbreaks, 1996-2005
Avian influenza in Asia, 1997
Nipah virus in Malaysia, 1999
West Nile virus in USA, 1999
Anthrax mailings in USA, 2001
SARS in Asia, 2002-2003
Monkeypox in USA, 2003
Notable Outbreaks and technological advances, 2006-present
Foodborne Outbreaks
Avian Influenza
Emergence of Chikungunya, dengue, yellow fever and Zika viruses
Crimean-Congo hemorrhagic fever, severe fever with thrombocytopenia, heartland viruses
Ebola in West Africa, 2014-2016

AIDS, the isolation of Hantaan virus as the cause of Korean hemorrhagic fever in Korea and epidemic hemorrhagic fever in China, and the discovery of hepatitis C virus were among newly recognized pathogens that set the stage for *Emerging Infections* and served as a prelude for the many new diseases that were to be discovered in the coming quarter century. Here we note some of the more significant new pathogens discovered and the notable outbreaks that led to advances in global policies and technologies to address these emerging threats (Tables 1 and 2).

Notable Emerging Infectious Diseases, 1992-1995

Hantavirus in the Americas, 1993. In May of 1993 an outbreak of a previously unknown disease was reported in the southwestern United States (2). This rapidly fatal illness was primarily seen among rural, previously healthy young men and after considerable investigation was determined to be caused by a novel virus later named Sin Nombre virus. This new virus was shown to be related to Hantaan virus, the cause of hemorrhagic fever with renal syndrome, previously found only in Asia and Europe. The disease caused by the new virus was named Hantavirus Pulmonary Syndrome (or now Hantavirus Cardiopulmonary Syndrome) (3). Patients typically suffered from acute shortness of breath and often died very rapidly, even when hospitalized (4). Hantaviruses are frequently associated with rodent hosts, and subsequent investigations found Sin Nombre virus to be harbored by wild deer mice (5). As studies continued, additional New World hantaviruses were discovered from a variety of rodent hosts and many of these new viruses were shown to cause similar life-threatening human infections. The discovery of a completely new group of viruses within the United States and throughout the Americas causing fatal human infections

drew considerable attention in the popular press and was an early validation of the concept of "Emerging Infectious Diseases."

Plague in Surat, India, 1994. Between August and October of 1994, an outbreak of bubonic and pneumonic plague struck the Indian city of Surat (6). Discovery of this historic disease in a major urban center of India triggered widespread panic and about 300,000 people left the city over the course of a few days. The resulting crisis led to flight cancelations and blockage of imports from India. The economic impact of the outbreak far exceeded the real human toll of a little over 50 deaths but it made a significant impression on the global community as clear demonstration of the potential magnitude of a public health crisis (7).

Hendra Virus in Queensland, Australia, 1994. A small outbreak near Brisbane, Australia in 1994 led to the death of a horse trainer and some of his horses and resulted in the discovery of Hendra virus (8). This was an absolutely new virus, completely unknown to science yet capable of killing both horses and humans (9). While the number of infections was not great, the fact that a novel virus had been discovered captured the interest of the general public and the scientific community. This was yet another example of an emerging infectious disease.

Ebola Hemorrhagic Fever, Kikwit, DRC, 1995. Ebola virus was first discovered in 1976 and was associated with a devastating outbreak in central Zaire (now Democratic Republic of the Congo) and nearly simultaneously in a second outbreak near the border between Zaire and Sudan (10, 11). The outbreaks were caused by distinct viruses now termed Ebola Zaire and Ebola Sudan, but both caused human disease with very high case fatality rates and little options for treatment. Following these original outbreaks, only isolated cases were reported until a major outbreak of Ebola Zaire occurred in Kikwit, DRC (12). The outbreak followed the pattern seen earlier with very high mortality rates, with an especially devastating impact on the healthcare infrastructure. Unlike the original Ebola outbreaks that occurred in remote locations with difficult access and poor communications, the Kikwit outbreak was reported in near real-time to a global audience (13). For the first time, the world could read about the unfolding tragedy and see photos from the actual outbreak site. As a result, the name Ebola became part of our vocabulary as the cause of one of the most dangerous diseases ever known.

Impact

These outbreaks, all well documented in the popular press, gave witness to the validity of the observations made in the NASEM report – that emerging infectious diseases were a part of modern life and that they demanded a new, more agile approach to their recognition and control. In the United States, the Centers for Disease Control and Prevention (CDC) had initiated a new program to implement the recommendations suggested in the NASEM report and one facet was to engage with the World Health Organization (WHO) in an effort to coordinate actions globally. The result was the development and acceptance of two World Health Assembly resolutions relevant to emerging diseases: WHA48.7 Revision of the International Health Regulations and WHA48.13 New, Emerging and Re-Emerging Infectious Diseases (14). Together these resolutions set in motion the creation of a new program to address emerging diseases by the WHO and the development of the revised International Health Regulations which was finally accepted in 2005 and implemented in 2007. These and other actions set the foundation for WHO's engagement in emerging infectious diseases that continues to this day.

Notable Infectious Diseases, 1996-2005

Nipah Virus in Malaysia, 1999. Nipah virus was first isolated as part of an outbreak of encephalitis and respiratory disease seen primarily in commercially raised pigs and in pig farmers (15). Nearly 300 human infections were seen with over 100 deaths and more than one million pigs were euthanized to control the outbreak (16). Nipah and Hendra viruses are closely related and studies of each found large flying fox (*Pteropus)* bats as the likely reservoir host (17, 18). Cases of Nipah are now recognized most years among rural residents of Bangladesh. The significance of this outbreak was not only the discovery of yet another new virus disease with the ability to kill both humans and domestic animals, but also the tremendous economic impact that the outbreak had on the agricultural sector.

West Nile Virus Introduced into the United States, 1999. West Nile virus is a mosquito-borne pathogen that has long been recognized to cause illness and occasional death in humans and animals in various countries of Asia and Africa, but until its introduction in Queens, New York in 1999, it had never been found in the Americas (19). West Nile virus found a very receptive environment in the United States with competent mosquito vectors and susceptible vertebrate populations. Over the course of the following decade the virus marched across the continental United States and expanded its range into Central and South America such that today it is found in most countries of the Americas. It has caused thousands of severe neurological cases in humans and hundreds of fatalities, mass mortality among birds, especially crows, and encephalitis in horses, all primarily in the United States but also throughout the Americas (20). The significance of this outbreak was its demonstration of the receptivity of new ecological environments to the introduction of exotic pathogens, the difficulty in controlling vector-borne diseases, and the significant impact that introduced pathogens can have on human health, wildlife and agriculture.

Anthrax Mailings, United States, 2001. Shortly after the terrorist attacks of September 11, 2001, a number of letters containing anthrax spores were sent to various individuals including political leaders and news organizations resulting in five deaths and 17 others infected (21). Anthrax had been previously weaponized as a biological warfare agent and its ability to cause serious and fatal disease following aerosol exposure was well known, but its intentional use as a terrorist weapon disseminated by the postal service was a stark warning of the potential misuse of modern biology (22). Here was an emerging disease of manmade origin. This event triggered a massive increase in spending in the United States to protect the nation against bioterrorism and drew global attention to the potential for misuse of dangerous pathogens. While the United States select agent program was already in place when the anthrax mailing occurred, the event nonetheless heightened the need for enhanced security surrounding laboratories handling especially dangerous pathogens.

Severe Acute Respiratory Syndrome (SARS), Asia, 2002-2003. In late 2002 rumors surfaced that an outbreak of severe respiratory disease was underway in southern China. Shortly after the start of the new year a physician who had treated such cases travelled to Hong Kong, stayed briefly in a Hong Kong hotel, became ill and was hospitalized and later died. He became the index case of one of the most devastating outbreaks in recent memory (23). A highly contagious new coronavirus was shown to be the cause of the illness and was named SARS virus and over the following several months over 8000 cases occurred with nearly 800 deaths (24, 25). In addition to being a completely new virus to science, this outbreak was noteworthy by the speed with which it was dissemi-

nated around the world, how cases emerged even in modern hospitals with excellent facilities and skilled clinical staff, and how unprepared the world really was to address a modern emerging infectious disease. Historic concepts of public health control such as quarantine and patient isolation had to be adapted to modern society.

Monkeypox Virus, United States, 2003. Monkeypox virus is related to smallpox and is found in Central and West Africa where it is maintained in a zoonotic cycle involving squirrels and other rodents, occasionally spilling over to infect humans (26). In 2003 just as the SARS pandemic was subsiding, a febrile illness with rash was seen in a Wisconsin child following a bite by a pet prairie dog purchased from a local pet vendor. Subsequent investigations found Monkeypox virus as the cause and over 70 human cases were ultimately identified throughout the Midwest United States. Extensive investigations of the prairie dog revealed a complex network of formal and informal sales of exotic pets linking the prairie dogs to recently imported Gambian pouched rats from West Africa (27). The significance of this outbreak was discovery of the extensive movement of wild animals collected in rural Africa which were easily moved to the United States where they were sold as exotic pets without any regard to the diseases they might be harboring. Subsequent policy changes were implemented to prevent the importation of some species of rodents from Africa and the sale and distribution of prairie dogs.

Impact

The outbreaks and epidemics of this decade, in addition to providing yet more examples of new diseases arising around the world, also brought to light several shortcomings in existing policies that hampered the ability of health officials to adequately address emerging infectious diseases of global importance. The SARS epidemic perhaps more than any other event demonstrated the severe economic toll that infectious diseases can impose on the global economy. Further, it highlighted the challenges of information sharing internationally, and the need for modern interpretation of mitigation strategies that allow quarantine of those exposed and isolation of infected patients suffering from a highly communicable disease. The monkeypox outbreak clearly demonstrated the importance of zoonoses to global health and the need for improved regulations to protect unsuspecting consumers from exposure to zoonotic pathogens. In 1999, prior to the anthrax mailings, the United States federal government by way of the CDC spearheaded the creation of the Laboratory Response Network (LRN), a defined network of laboratories within the United States and abroad with the capacity to rapidly respond to biological, chemical, or radiological threats via timely diagnostic testing and secure result notifications. One of the early successes of the LRN was its role in aiding with the rapid identification of *B. anthracis* from the 2001 anthrax letters. The anthrax mailings were nonetheless a stark reminder of the power of pathogens as potential weapons and the need for further safeguards surrounding the safe and secure handling of these agents. This decade of emerging infections also resulted in the final agreement on the revised International Health Regulations which placed new emphasis on WHO's role in outbreak response, improved public health laws to govern outbreak interventions taken by health officials, and increased regulation of the exotic pet industry.

Notable Infectious Diseases, 2006 - Present

Foodborne Outbreaks. With the globalization of the food supply has come more complex outbreaks of foodborne illnesses. Major

outbreaks caused by hepatitis A virus, toxigenic *E. coli*, listeria and others have been publicized in the popular press and resulted in many human infections (28). The commercial food producers, importers, suppliers and consumers have worked closely with public health officials to improve production techniques, better track products and recognize outbreaks earlier so that effective interventions can be made rapidly and future risks mitigated.

Avian Influenza. Influenza viruses, especially influenza A viruses, are the classic example of an emerging infectious disease. Influenza A H1N1 and H3N2 viruses circulate, along with influenza B viruses, among humans every year. These viruses evolve through antigenic shift and drift to create new strains that may avoid existing protective immunity among humans and may cause pandemics as was seen during the 2009 pandemic of a novel influenza A H1N1 virus that rapidly spread around the world over the course of a few months (29). Lurking silently is a vast array of other zoonotic influenza viruses that may cause disease and death in animals and may mutate or recombine to form a virus transmissible among humans. For the past decade and more there has been growing concern about the possibility of a major pandemic of influenza and considerable investment has been made to improve global surveillance of influenza viruses circulating in both humans and animals, promptly stomp out emerging outbreaks among animals, and improve national preparedness in the event of a pandemic. The rapidity of spread of the 2009 novel H1N1 influenza A virus clearly demonstrated weaknesses in global preparedness efforts and the need for continued work to improve global surveillance, enhance vaccine production and distribution, and revise preparedness planning.

Chikungunya, Dengue, Yellow Fever and Zika Viruses. These viruses share the common characteristic of being transmitted by the urban mosquito vector, *Aedes aegypti*. Once nearly controlled in many parts of the world, *Ae. aegypti* is now found in most urban centers of the tropics and subtropics around the world (Figure 1). The mosquito has adapted to live in close association with humans and is a competent vector of many viruses (30). Introductions of "new" viruses such as Chikungunya and Zika into New World habitats have resulted in thousands of human infections and the unfolding crisis of children being borne with severe disabilities following Zika infection during pregnancy (31). This has occurred against a backdrop of periodic major epidemics of any of the four dengue serotypes now circulating in many communities. The combination of urban growth, rapid travel, poverty and eroding sanitation coupled with the mosquito's ability to easily co-exist with humans has resulted in difficult challenges in vector control and disease prevention. The threat of not only newly emerging viruses to cause vector-borne outbreaks, but also the risk of urban transmission of often fatal yellow fever virus represents a major challenge to health in many corners of the world.

Crimean Congo Hemorrhagic Fever, Severe Fever with Thrombocytopenia, Heartland and Powassan Viruses. Similar to the growing importance of mosquito-borne pathogens, the dramatic rise of tick-borne pathogens is concerning. New viruses such as severe fever with thrombocytopenia virus, a tick-borne virus recently discovered in China, Heartland virus recently found in the United States, and classic viruses such as Crimean Congo hemorrhagic fever virus and Powassan virus are all examples of viruses that are either newly discovered or that have been known for many years but are only recently gaining the attention of public health officials due to their increased incidence and serious disease following infections (33). It is not clear if the rise in tick-

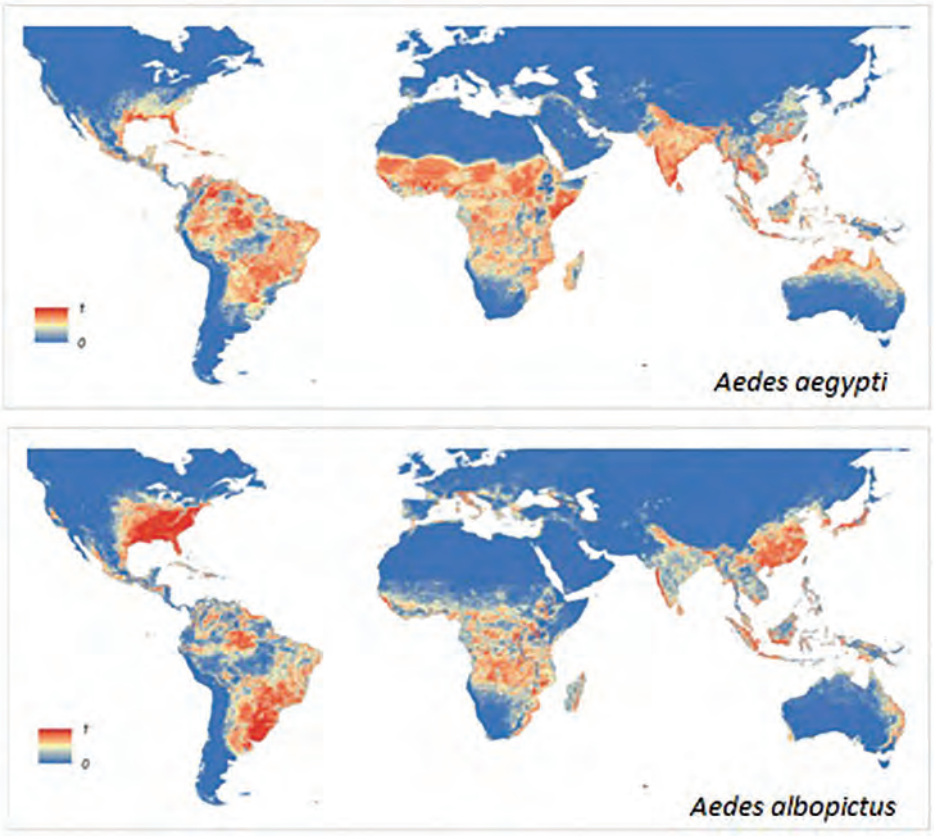

Figure 1. Global distribution of *Aedes mosquitoes* of public health importance. Estimated *Aedes aegypti* and *Aedes albopictus* distribution globally based on high (red) or low (blue) probability of habitation (32).

borne pathogens is the result of improved surveillance and better laboratory capabilities, or an increase in the abundance of ticks and their pathogens due to environmental changes and human population growth. Regardless, it is clear that tick-borne diseases have increased in prevalence and are likely to continue to do so, undoubtedly leading to the discovery of additional new emerging infectious diseases.

Ebola Virus, West Africa, 2014-2016. The Ebola outbreak of 2014-2016 was the nightmare scenario many public health officials had dreaded—a highly pathogenic, easily transmissible disease introduced into an urban center lacking adequate health care infrastructure or the ability to rapidly and effectively interrupt transmission (34). The outbreak resulted in over 28,000 cases and over 11,000 deaths, most of which occurred in Liberia, Sierra Leone and Guinea. Cases were, however, exported to surrounding countries in Africa and to various distant countries including the United States, the United Kingdom, and others. The outbreak clearly demonstrated many of the concerns originally raised in the NASEM report—the importance of zoonotic infections and the risks they represent to human health, the importance in global travel as a potential disseminator of infections, the impact of population growth and urbanization coupled with poverty, and the need for global coordination in responding to such outbreaks (Figure 2).

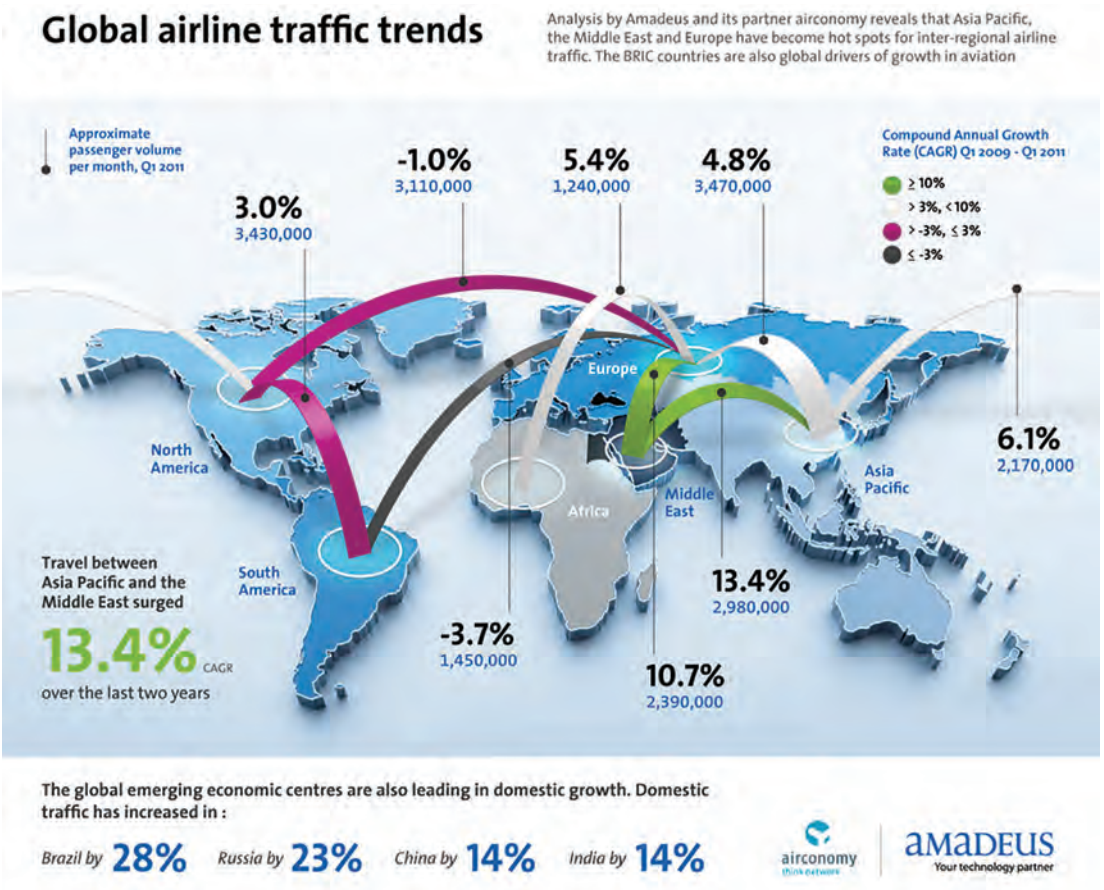

Figure 2. Global Airline Traffic Trends (35).

Impact

These and other events involving emerging infectious diseases have only served to heighten awareness amongst health officials and the general public that "new" diseases will continue to occur with some regularity. The rapidity of their spread has been facilitated by our modern international travel network that allows anyone to reach almost any place in the world in less that the incubation period of virtually all infectious diseases (Figure 2). This was dramatically demonstrated when a traveler from West Africa entered into the United States while asymptomatically incubating Ebola virus (36). The patient was carefully screened on arrival and found free of symptoms, yet a few days after landing in Dallas, Texas, he was seen at a local hospital, sent home initially, and only later hospitalized and diagnosed as an Ebola patient. Unfortunately, the patient died and two nurses providing care for him were infected themselves. The entire event reinforced the adage that an infectious disease outbreak anywhere is a threat everywhere.

The increased attention to the risk of pandemic influenza resulted in significant investments to improve global surveillance of influenza in several ways, including the establishment of national influenza centers in many countries, the rapid characterization of circulating viruses, and the sharing of surveillance information with the WHO to improve the composition of seasonal influenza vaccine. In addition, much greater awareness of avian influenza resulted in vastly improved global information sharing and

Table 2. Global impact and response to outbreaks of emerging infectious diseases, 1992 to the present

1992-1995—Validation of the concept of "Emerging Diseases"
Hantavirus in the Americas, 1993
Discovery of new viruses and diseases
Plague in Surat, India, 1994
Significant direct and indirect economic impact of outbreaks
Hendra virus in Australia, 1994
Emphasized reality of emerging infectious diseases
Demonstrated the importance of human-animal interface →"One Health" concept
Enhanced the credibility of CDC and WHO initiatives on Emerging Infectious Diseases
Ebola Hemorrhagic Fever, Kikwit, DRC 1995
Importance of press coverage; raised global awareness of serious outbreaks
Outcome
The world began to take notice of outbreaks
1996-2005 — Global Recognition and Impact
More new viruses—further confirmed the concept of Emerging Infectious Diseases
Demonstrated the ecological receptivity to introduction of new pathogens in other countries
Demonstrated the enormous economic impact of outbreaks
Witnessed rapid spread internationally
Identified risks of exotic imported "pocket pets"
Showed the intentional mis-use of biology as a weapon
Outcome
Revision of International Health Regulations initiated; revisited quarantine laws; modeling tools for outbreaks gained popularity
Regulations created to control import of wild animals as pets
Biodefense programs dramatically increased
Growth of Select Agent Program
2006- present—Increased Recognition of Threat and Heightened Response
Heightened awareness of Emerging Infectious Diseases globally
Highlighted the importance of global food-chain in food safety and introduced new technological advances for their control
Demonstrated the rapid international spread of disease
Showed the real threat of global pandemic influenza (and other respiratory transmitted diseases)
Exemplified the importance of vector-borne diseases—mosquitoes and ticks
Raised international concern over dual use research and gain of function studies
Outcome
PulseNet Established—whole genome sequencing incorporated in surveillance
FDA,USDA, European Food Safety Authority and others more active involvement; food recalls
National Influenza Centers expanded and global influenza surveillance strengthened
New approaches in vector control explored—gene drive, Wolbachia, others
National Science Advisory Board on Biosecurity (USA) created; Select Agent program strengthened
WHO-Global Outbreak Alert and Rresponse Network, Medecines Sans Frontieres and others increase epidemic preparedness and response (ongoing)
International Health Regulation revised and implemented; Joint External Evaluations started to assess national capacity
Global Health Security Agenda initiated
Increased role of philanthropy in global public health—Gates Foundation and others

coordinated control efforts especially in China and other countries in Asia where avian influenza is prevalent. Greater recognition is now being paid to vectors and vector control, with modern biotechnology being used to engineer lethal mutations in mosquito vectors and the first real novel approaches to the control of *Ae. aegypti* mosquitoes in decades (37). Unfortunately, similar progress has yet to be made in the control of vector ticks.

The past decade of emerging infections has served to highlight the synergism of human, animal and environmental factors in impacting public health, resulting in the creation of the One Health initiative in 2008. The initiative recognizes that the majority of new emerging infections are zoonotic and works to foster multidisciplinary collaborations in order to advance the health of all species and their environment.

Technological Advances in the Past Quarter Century

In the early 1990s email was starting to be more widely used internationally, the internet was growing, and the internet-based disease reporting system ProMED, the Program for Monitoring Emerging Diseases was launched. Social media was not yet popular, but it was clear that a revolution in information sharing was underway and that it was increasingly difficult for outbreaks of disease to escape international recognition. This trend only increased in ensuing years and it became apparent that the International Health Regulations of 1969 were no longer appropriate for the modern, interconnected society and, as indicated above, the process to revise the regulations began in 1995, was approved internationally in 2005, and the new regulations are now in place. Many changes resulted from the revisions, including placing greater demand on countries to build their own core capacity to recognize and respond to disease threats and providing WHO with greater flexibility to respond to outbreaks of international significance. This process is still evolving, but it is clear that major outbreak responses today involve a wider array of players including Medicine Sans Frontier (MSF), the European Centers for Disease Control (ECDC), the US Centers for Disease Control and Prevention (CDC) and many others. Further, countries like China have established their own internal CDCs that provide much improved surveillance, diagnosis, and response to emerging infectious diseases. Finally, the appearance of major philanthropic organizations such as the Bill and Melinda Gates Foundation have greatly impacted global health through targeted investments and capacity building internationally.

As indicated, today many of us enjoy fresh fruits and produce any time of the year, independent of the seasons. This is the result of a growing international network of growers and rapid transportation. Many advances have helped to improve our food supply and distribution network. Parallel advances have been made in technology such as PulseNet and more recently genomic sequencing that allows public health officials to rapidly characterize pathogens and track foodborne outbreaks nationally and internationally. Similarly, advances in diagnostic technology have transitioned from classic culture techniques to molecular-based identification and genomic "finger printing" of pathogens, often available in hours rather than days to weeks. In some cases, these assays are even available as point of care tests.

Over the past quarter century a much greater awareness has evolved regarding the importance of biosafety and biosecurity surrounding pathogens, especially those like anthrax that were previously weaponized. One result was the creation of the select agent program in the United States, designed to limit the distribution of dangerous pathogens to known entities and appropriately trained and screened individuals. This has consolidated research on dangerous pathogens and spurred the construction of

biocontainment laboratories in the United States and abroad. The proliferation of biocontainment laboratories in theory allows research to be conducted more safely and securely; however, the cost of operations and the complexity of the facilities may become burdensome. Further, experimentation that involves changing the characteristics of pathogens such as the recent avian influenza gain-of-function (GOF) studies may result in added risk or even the threat of accidental release and creation of a pandemic (38). Modern biotechnology has also been used to recreate an extinct virus, horsepox, an accomplishment that raises serious questions about the possible construction of more dangerous viruses such as smallpox (39). Clearly many, many important advances are being made through biotechnology that benefit all humankind; however, the risk of misuse or accidental release is an unwelcome cost that cannot be ignored.

Where Are We Going? Challenges and Opportunities of the Future

No one can foretell the future, but one thing is clear: The emergence of new pathogens that we have witnessed over the past quarter century will continue to occur, only faster and with a bigger impact. Faster because we and our "things" are moving faster, more frequently and further distances than ever before. Bigger because there are more of us, we live in bigger urban centers, and we coexist closer together than at any time in history. Our warming world will impact us in many ways yet to be fully realized, but it's clear that vectors of disease are likely to be more abundant and more widely distributed. Advances in biotechnology and synthetic biology will create new products and improve health, perhaps even eliminating some devastating inherited diseases for generations to come. We would be wise, however to be cautious as we employ these new tools in biology.

What are the greatest threats to become the next emerging disease? It's impossible to predict, but four areas deserve our increased attention (Table 3):

Respiratory Transmitted Viruses. We know that seasonal influenza viruses are continually evolving and that every season we are faced with the threat of a new strain. We have also monitored avian influenza, watching for the emergency of a strain easily transmitted among people. These clearly should remain a focus of our attention, but we should not ignore the fact that there are other virus diseases that are easily transmitted by the respiratory route and may emerge at any time. The coronaviruses are a prime example based on our experiences with

Table 3. Potential Threats to be the Next "Emerging Diseases"

Respiratory Transmitted Viruses
Influenza A viruses, both seasonal and avian influenza—H7N9 currently greatest concern
Coronaviruses and other respiratory infections yet to be recognized
Vector-borne viruses
Viruses transmitted by *Ae. aegypti* of special concern—Yellow fever (due to shortage of vaccine); perhaps Mayaro virus; other vectors following the West Nile model—Japanese encephalitis
Tick-borne pathogens—several "new" viruses recently discovered with others likely as temperatures warm and tick ranges expand; rickettsial diseases; Lyme disease
Repeat of Ebola scenario
Easily transmitted dangerous pathogen in over-populated areas with poor health infrastructure (Marburg, Ebola Sudan)
"Bio-Errors"
Laboratory generation of extinct viruses; gain of function experiments; mis-use of synthetic biology

SARS, and the smoldering situation with MERS in the Middle East.

Vector-Borne Viruses. As mentioned already, the re-infestation of many communities where *Ae. aegypti* had been eliminated previously and its introduction into new geographic areas, coupled with its remarkable ability to thrive in urban and suburban environments, sets the stage for potential widespread transmission of a number of well-known arboviruses like dengue and yellow fever viruses as well as others not commonly known but potentially of great public health importance. Chikungunya and Zika viruses have emerged recently, and many experts think that Mayaro virus may be next (40). *Ae. albopictus* shares many of the same vector characteristics as *Ae. aegypti* but has a wider host range and geographic distribution and may serve as an important secondary vector for many arboviruses. The current shortages of yellow fever vaccine are especially disconcerting given the enzootic nature of yellow fever virus in many tropical countries and the potential for large urban outbreaks. Many species of ticks are efficient vectors of disease and in recent years we have seen several new viruses identified, some of which like severe fever with thrombocytopenia virus recently discovered in China, are highly pathogenic and much more widely distributed than originally thought. Other well-known viruses such as Crimean-Congo hemorrhagic fever virus have increased dramatically in some parts of their distribution. Tick abundance and the factors that affect tick-borne diseases are not well understood and they are likely influenced by a number of factors including land use, human encroachment and perhaps changing environmental conditions. Regardless of the core drivers, it is likely that we will see additional new tick-borne diseases in the years to come.

Repeat of the Ebola-West Africa Scenario. The conditions that allowed Ebola virus to cause the horrific outbreak witnessed in 2014-16 are little changed today as compared to when the first case was introduced into the local urban settings. We know that highly virulent filoviruses continue to exist in silent zoonotic transmission cycles in nature in tropical Africa and perhaps elsewhere, and the massive urban centers across Africa with limited healthcare infrastructure and widespread poverty grow annually. The level of international trade and travel between African urban centers continues to expand, and while national governments and the international community have attempted to improve recognition and response capabilities in preparation of future outbreaks, there remains much to be done. The need for timely detection and control of the severe, life-threatening diseases caused by deadly pathogens such as Ebola is great and progress remains limited.

"Bio-Errors". Tremendous progress has been made through biotechnology to protect people from disease and improve the quality of life for millions around the world. This progress will undoubtedly continue; however, as technology improves and becomes more widely accessible, there is a very real possibility that mistakes may be made that could result in the creation or release of a known or novel pathogen that could cause serious disease or even start a pandemic. One significant risk are so-called "Gain of Function" studies that interrogate the molecular basis for transmissibility of pathogens with restricted host distribution like avian influenza. The proliferation of biocontainment laboratories around the world may help reduce the risk to the public, assuming that they are well maintained and employ appropriate safety and security precautions. Nonetheless, we should carefully consider the risks and benefits of studies that potentially modify the characteristics of microbes that may cause disease in humans or animals.

How do we ultimately address the emerging infections of tomorrow? Coordination

of international responses and the rapid mobilization of resources will be paramount. In recognition of this, the WHO established the Global Outbreak Alert and Response Network (GOARN), a collaboration of institutions worldwide committed to rapid and coordinated responses to emerging infections. This and other WHO initiatives may serve to better coordinate the international response to future outbreaks, while continued efforts to build national and local capacity will be important to ensure rapid recognition of emerging threats and implementation of targeted interventions. In addition, advancement of research on emerging pathogens will be essential in combatting the next major public health threat. Importantly, in recent years, the number of high containment (biosafety level 3 and 4) laboratories throughout the world has increased significantly. Despite inherent concerns regarding best practices and safety regulations, these new laboratories may enable a larger swath of the population to pursue important research that may prevent emerging infections from becoming global threats.

Conclusion

As our world continues to evolve in the direction of advancing technology, human expansion, and climate change, so must our global discourse and response to the emerging infectious diseases that will undoubtedly continue to impact our world in the coming decades.

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Historical article

Review article
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Pearls of Neonatal Intertrigo in Ancient Greek and Byzantine Medicine

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Key words: Ancient Greece ▪ Byzantium ▪ Intertrigo ▪ Botanology ▪ Paediatric dermatology.

Objective. Confirmation of knowledge of neonatal intertrigo in ancient Greek and Byzantine medicine. Method: A search of Thesaurus Linguae Graecae was conducted with the following terms as key words, *"syggama"*, *"xyggauma"*, *"paratrimma"* and *"ektrimma"*. Results: Ancient Greek medico-philosophers introduced therapeutic measures based upon herbs and minerals, while a similar therapeutic approach was also used by the Byzantines. Hippocrates of Kos (460-377) was among the first to introduce written instructions, also proposing preventive treatment with palliative and aromatic herbs. Cataplasms, thalassotherapy, and fumigation were used, combined with hygienic measures in the affected area. Chalk powder was also prescribed to absorb moisture. **Conclusion.** The main principles in the treatment of intertrigo remain the same, celebrating the ancient Greeks' methodology and rationalism.

Introduction

Neonatal intertrigo is an inflammation of the skin folds, of irritant or infectious aetiology. Neonates and infants are more predisposed towards intertrigo as they have abundant subcutaneous tissue which prevents the folds from airing out. Prolonged contact with an irritant, mainly detergents, urine, faeces and fragrances, affects the skin properties and enhances permeability to exogenous materials, thus increasing transepidermal permeability and susceptibility to friction. Therefore, friction between the skin folds, coupled with maceration, due to reduced evaporation of sweat and overgrowth of bacteria (*S. aureus*, *Strep. pyogenes*) and fungi (*Candida albicans*), contribute to the development of erythema, pruritus, maceration and erosions, which may be quite persistent. Therapeutically, gentle cleaning, keeping the affected area dry, and topical barrier agents (zinc oxide paste) can be very effective. In some cases, imidazole lotions and antibacterial agents, such as mupirocin cream, and topical corticosteroids may also be administered. However, the importance of prevention, in terms of cleanliness, dryness and use of protective barriers, should be always stressed (1-3).

Intertrigo, as a term, comes from the Latin words *"inter"* which means "in between"

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and *"terrere"* which means "to rub" (4). It was a well-recognized affliction in Ancient Greece, as Hippocrates (460-377) and his followers were among the first authors to describe it, in the *"Corpus Hippocraticum"* (5). The terms used by the Greek medico-philosophers in antiquity were *"syggama"* (Greek: σύγκαμα) or *"xyggauma"* (Greek: ξύγκαυμα), which means *"friction burn"*, *"paratrimma"* (Greek: παράτριμμα), which means *"rubbing between two surfaces"* and *"ektrimma"* (Greek: ἔκτριμμα), which means *"a lesion caused by friction"* (6).

With the goal of discovering the knowledge of the ancient Greeks and Byzantines regarding intertrigo, our study gathers all the text fragments found in the TLG (*Thesaurus Linguae Graecae),* a digital collection of surviving texts written in Greek, after a search using those terms as key words.

Neonatal Intertrigo in Ancient Greek and Byzantine Dermatology

Neonatal intetrigo has been recognized since antiquity, and ancient Greek physicians proposed various therapeutic approaches (6). In the 5th century BC, Hippocrates and his followers first mentioned the importance of prevention, and proposed cataplasms based on a mixture of aromatic herbs *(On Diseases, 4th Book)* (7). Furthermore, he introduced seawater bathing, or steam bathing (boiled seawater) for alleviation of pruritus *(On the Use of Liquids)* (8). In his opinion, precautionary measures should be administered soon after birth to avoid intertrigo (Greek fragment: *Επήν τα παιδία γέννηται, ψωμίζουσιν αυτά αι γυναίκες τα αυτά φάρμακα... και μη ξυγκαυθή*) (7).

Centuries later, the distinguished Greek pharmacologist and leading expert in therapeutic botanology, Dioscorides (40-90) proposed, for treatment of intertrigo, a mixture of *Myrtus communis*, *Lycium barbarum*, *Plumbago capensis*, chalcopyrite, white lead, and litharge. A palliative watery poultice was used as a cataplasm; aromatic oils, or even a powder of minerals and dried natural herbs were also applied (9). Dioscorides' view was adopted by Galen (130-201), who was considering litharge to be an amazing *"remedy for the human flesh"* (Greek: σαρκωτικό) *(On the Composition of Drugs According to Places)* (10). In his turn, the Byzantine physician Oribasius (320-400), having mentioned both Dioscoride's and Galen's views, suggested that intertrigo lesions *"should be covered with dry Myrtus, Cyperus Rotundus and rose hip, all stirred with some aroma"* (Greek fragment: *Τα δε κατά μηρούς εκτρίμματα μυρρίνη ξηρά διαπάσσειν και κυπέρω και ρόδοις προσμίγουσάν τι των αρωμάτων*) (11). The famous Byzantine physician and medical writer, *Aetius of Amida (502-575), proposed exactly the same treatment (Greek fragment: Μυρσίνην ξηράν λείαν επίπασσε, η κύπερον μετά ρόδων*) (12). Paul of Aegina (625-690), whose paediatric work was based upon Oribasius' treatise, also had the same opinion. According to his work, he proposed the topical application of *"Balaustium"* (Greek: *Βαλαύστιον*, Latin: *Punica granatum*, *varietas plena major*), the double flowered pomegranate tree, a plant with astringent properties in its bark, leaves and blooms, to cure intertrigo (13).

Oribasius and the Arab-Islamic physician Al Rhazi (854-925) mentioned that Soranus of Ephesus (98-138), the founder of obstetrics and gynaecology, and an expert in paediatrics, had dealt with neonatal intertrigo in his treatise entitled *"On the care of the small child"* (Greek: Περί κομιδής παιδιού, Arabic: Tadbir al atfal) (11, 14). In his turn, Avicenna (980-1037), adopted and proposed the ancient Greek treatment for intertrigo in his masterpiece, *"The Canon of Medicine"* (Arabic: *al-Qanun fi al-Ṭibb*) (15, 16). Centuries later, the Swiss physician and alchemist Philippus Aureolus Theophrastus Bombastus Von Hohenheim (1493-1541),

known as Paracelsus, mentioned in his work that the ancient Greeks used herb *Helleborus niger* (Ranunculaceae family), a well-known plant in antiquity, to treat skin irritations such as intertrigo (17). A reference was also made to its *rose* like flowers, while no other such citation is available.

Discussion

Ancient Greek medico-philosophers divided cutaneous diseases into *psoriasic*, *leprotic* and *leichen*. The term *psora* referred to itching and erythema, depicting moist pustular ulcerated conditions and eczematous like eruptions. The term *lepra*, from the Greek words *"lopos"* (Greek: λόπος, the epidermis) and *"lepo"* (Greek: λέπω, remove the outer layer), referred to scaly conditions, while *leichen* represented skin growths held on by adhesion to the base (5, 18). So, intertrigo was classified as a "psoriasic" dermatological disease and physicians proposed the treatment of so called *neonatal psora,* as soon as it appeared (6).

Since environmental interaction with the human's body homeostasis has been known from the Hippocratic era (6), there is a great possibility that Soranus of Ephesus and Paul of Aegina differentiated intertrigo from allergic and irritant contact dermatitis, which usually presented with more intense pruritus and signs of eczema (from the Greek words *"ec"*: over, *"ze"*: boiling, and *"ma"*: result of, thus describing a more superficial reaction), a more severe condition than the simple erythematous lesion of intertrigo. Thus, in cases of eczema, they suggested *"hot pads with a rich suffuse of boiled olive oil are useful, mixed with a small quantity of melted wax in order for the mix to be thicker, for longer adherence"* (19, 20).

A series of herbs were harnessed by the Greeks in antiquity to treat neonatal intertrigo (Figure 1).

Among them, *"Myrtus communis"* (Greek: Μύρτος ο κοινός) of the family *"Myrtaceae"* represented the main ingredient for preparations against cutaneous affections. It is an aromatic evergreen perennial shrub or small tree, with small foliage and deep fissured bark. Its name, *"Myrtus",* signifies a common plant growing in groups. Myrtle occupied a prominent place in the writings of Hippocrates, Pliny (23-79), Dioscorides, Galen and the Arabian writers. According to them, the leaves and fruit were therapeutic for several diseases, including intertrigo (21-23). Nowadays, Myrtle is known to have antibacterial and antifungal properties, which exhibit remarkable activity against several fungal strains (24). *Cyperus Rotundus,* (Greek: κύπερος) of the *"Cyperaceae"* family, was used from the Mycenaean era as a purifying agent, a skin reviving, healing factor, and herb regulator of the body's humours (blood, phlegm, yellow bile, black bile) (13, 25). It is now known that *Cuperus* has antibacterial activities against *Staphylococcus epidermidis, Bacillus cereus, Pseudomonas aeruginosa, Escherichia coli* (26). *"Lycium barbarum"* (Greek: Λυκόμουρο, from the *ancient* southern Anatolian region of Lycia) was also widely used by the ancient Greeks as an anti-inflammatory agent (9). Flavonoids isolated from *Lycium barbarum* fruits have anti-inflammatory and anti-angiogenic effects (27). "*Plumbago capensis*" (Greek: μπλε Γιασεμί), of the *"Plumbaginaceae"* family, was used for the wood's healing capacities, which are appreciated even now, as well as its antibacterial properties, especially against *Staphylococcus aureus* (28). Furthermore, some studies mention its strong antifungal activity, equivalent to that of voriconazole (29). *Rose hip* (ancient Greek: ρόδον, Greek: κυνόροδον, Latin: rosa canina) is today a product in cosmetics, included not only among the fragrance substances but also in skin conditioning agents (30). On the other hand, *Punica granatum*

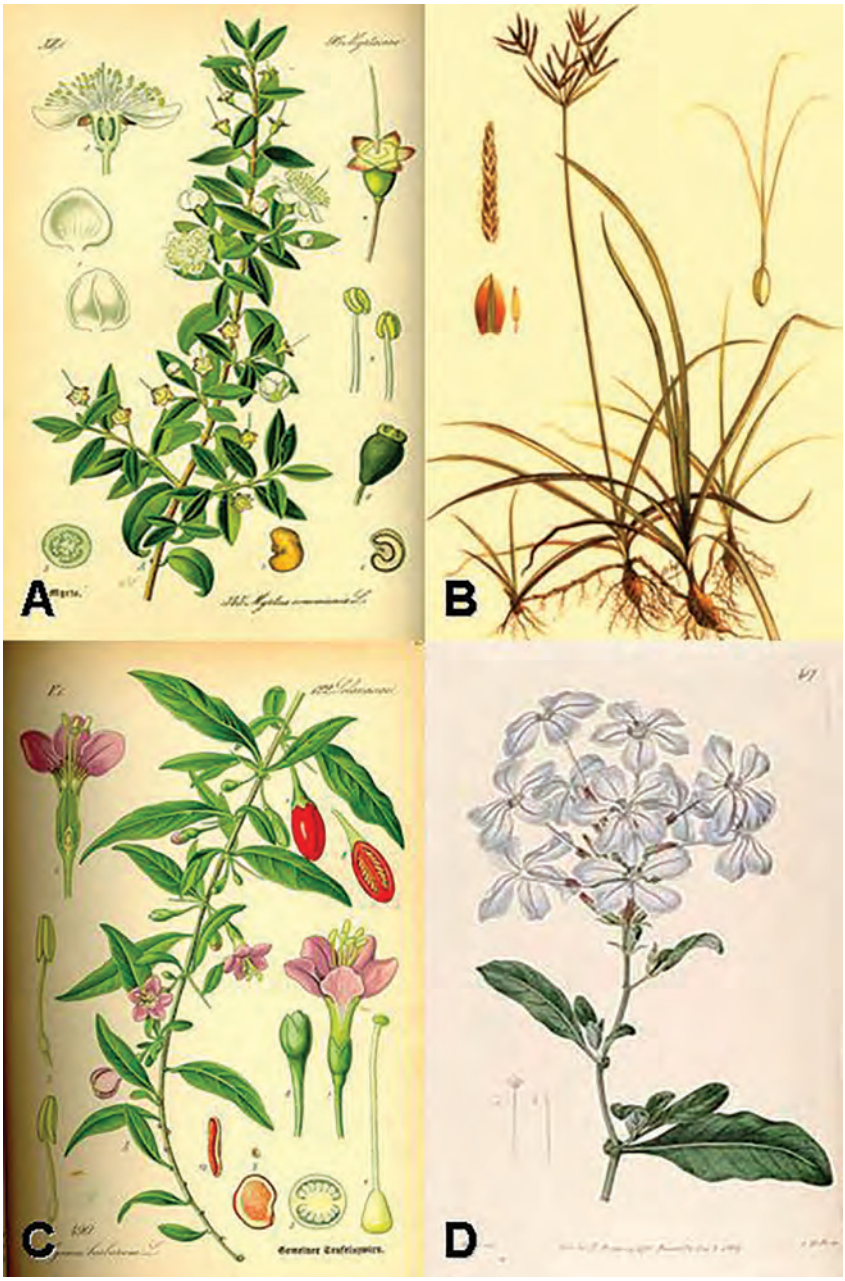

Figure 1. A: *Myrtus communis*, B: *Cyperus Rotundus*, C: *Lycium barbarum*, D: *Plumbago capensis*. A, C: colloured illustrations, *Flora von Deutschland*, Osterreich und der Schweiz, 1885, B, D: colloured illustrations, *Mills C*, Hortus Camdenensis, 1848.

is proposed as an anti-infection and anti-worm killer in modern female cosmetics (31). Meanwhile, "Heleborus niger" (Figure 2) for the ancient Greeks was a herb widely used against mild and severe skin diseases, from intertrigo to leprosy (32).

Strangely, the perennial plant is nowadays considered to be poisonous, while especially its roots may cause a skin irritation and blistering. In medieval Europe it was used in a small dosage as an anti-parasite drug (33). There is the possibility that there was another

Figure 2. Helleborus niger, Kräuterbuch, Actaea spicata, 1914.

species in Greek antiquity, less irritating than the "*Helleborus niger*" noted by Paracelsus, or it was perhaps used in poultices, or after some process which could have altered its side effects. It seems that Greek physicians in antiquity, experts in botanology, observed nature and the properties of herbs, used them to treat intertrigo in the form of

Table 1. Therapeutic ingredients used in ancient Greek and Byzantine dermatology and their current use.

No	Ingredient	Ancient Greek and Byzantine dermatology	Modern dermatology
1.	Myrtus communis	Skin therapeutic agent	Antibacterial Antifungal
2.	Cyperus Rotundus	Skin purifying agent Skin reviving and healing agent Humour regulator	Antibacterial
3.	Lycium barbarum	Anti-inflammatory	Anti-inflammatory
4.	Plumbago capensis	Healing wood capacity	Antibacterial Antifungal Repairing skin liaisons
5.	White lead	Base in powders Oil ingredient Skin healing agent Skin whitener Hair colouring	Basic acetate salt of lead as an ingredient in Water Soft Aluminium Men's hair colouring
6.	Litharge	Skin healing agent Tumours' lytic activity	-
7.	Thalassotherapy	Skin purifying and palliative agent	Hypertonic solution of sea minerals
8.	Chalk powder	Base in powders Moisture absorbent Astringent agent Cosmetics (eye colouring)	Talc ingredient

cataplasms, and practised dermatology in an almost scientific manner.

Thalassotherapy, introduced by the ancient Greeks, is a method still in use. A hypertonic solution of sea water penetrates skin lesions. Trace elements of magnesium, potassium, calcium, sodium and iodide found in seawater are believed to be absorbed through the skin. Thalassotherapy was applied in various forms: as showers of warmed sea water, inhalation of sea fog, or fumigation (34).

White lead powder was completely abandoned during the Byzantine era due to several deaths provoked by slow lead poisoning, a situation already known at least during the age of Pliny (23-79), and it was replaced by chalk powder, a soft, white, porous, sedimentary carbonate rock, a form of limestone, composed of the mineral calcite, well-known for its use in cosmetics in Greek antiquity (35). This powder, believed to have astringent properties, also had the ability to absorb moisture locally, but was also used as a base for the preparation of cataplasms. It was used extensively in the Byzantine era in the treatment of intertrigo, skin ulcers and burns (6, 36-38). Litharge (lead monoxide) was used as a lytic agent in tumour formations, and as a healing agent in skin ulcerations and intertrigo. Dioscorides believed that it also had the property of promoting cicatrisation, and it was also proposed as an anti-wrinkle agent and skin whitener in cases of melasma (9, 13, 39-41). Surprisingly, honey and olive oil, which were used in several cosmetic and skin preparations in antiquity, were completely neglected in the treatment of intertrigo (6). Finally, it is worth mentioning that the ancient Greek and Byzantine physicians' treatment for intertrigo (Table 1) was adopted in the medieval period by the Orthodox Monasteries in Russia, which operated as health centres (42).

Conclusion

The most significant medico-philosophers of Greek antiquity mentioned neonatal intertrigo, and suggested herbal and mineral based therapeutic approaches, thus proving not only their knowledge of pharmacology, but also their rationalism in the treatment of skin diseases. Similarly, preventive and curative measures for this type of dermatitis, initially mentioned in ancient Greek medical texts, continued in the Byzantine era and have surprisingly survived until the present day in the treatment of intertrigo.

What is already known on this topic

Intertrigo primarily affects neonates and infants. Neonatal intertrigo is a well studied entity, known since antiquity. Its causes, symptoms and treatment have all been adequately researched.

What this study adds

Our knowledge concerning intertrigo in Greek antiquity is somehow vague. This historical review gathers and sheds light on the available knowledge in the treatises of ancient Greek and Byzantine medico-philosophers. A comparison between ancient and modern treatment surprisingly reveals strong similarities.

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Historical article

Review article
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The Hippocratic Method for the Reduction of the Mandibular Dislocation, an Ancient Greek Procedure Still in Use in Maxillofacial Surgery

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Mandibular dislocation remains an acute medical condition in maxillofacial surgery. Since the Hippocratic era its reduction has been performed exactly in the same way it is now performed in modern surgery. We report an example of an elderly female patient with bilateral mandibular dislocation, who came to our department and was treated under sedation using the Hippocratic bimanual intraoral technique by two medical personnel, depicting the timeless usage of the method. Although some characteristics of the procedure were wrongfully attributed to others, such as Barton and Lewis, after a thorough examination of the ancient Greek medical texts, its originality should in fact be attributed solely to the Hippocratic School of Medicine. Furthermore, we recommend the use of the term "mandibular dislocation" instead of the "temporomandibular joint dislocation", as a more accurate medical definition.

Introduction

Although opinions concerning knowledge of anatomy in the Hippocratic era are controversial, Hippocrates and his followers were the first to introduce clinical anatomy. Even though post mortem examination was strictly prohibited, both by law and religious ethics, the Hippocratic school of medico-philosophers acquired sufficient experience to understand the human body's skeletal system, and described a series of bone fractures and dislocations. Apart from simply noting bone and joint disfigurations, they proposed treatment and palliation methods. their anatomic knowledge is revealed in "Corpus Hippocraticum" and especially in the treatises "Μοχλικός" (On the Instruments of Reduction, or Mochlicon), "Περί Άρθρων" (On Articulations), "Περί Αγμών" (On Fractures), "Περί των εν Κεφαλήν Τρωμάτων" (On Injuries of the Head), and "Περί Οστέων φύσιος" (On the Nature of the Bones) (Figure 1) (1). Among the plethora of orthopaedic interventional treatment procedures introduced by the Hippocratic School, stands the method for reduction of mandibular dislocation (MD) (2).

MD is nowadays an uncommon pathology, constituting a pathophysiological joint condition presenting oral and maxillofacial surgeons with a challenge in its management (3). The condyle of the mandible articulates bilaterally in a concavity, known as the glenoid fossa, or simply the mandibular fossa. Its dislocation, when the condylar process

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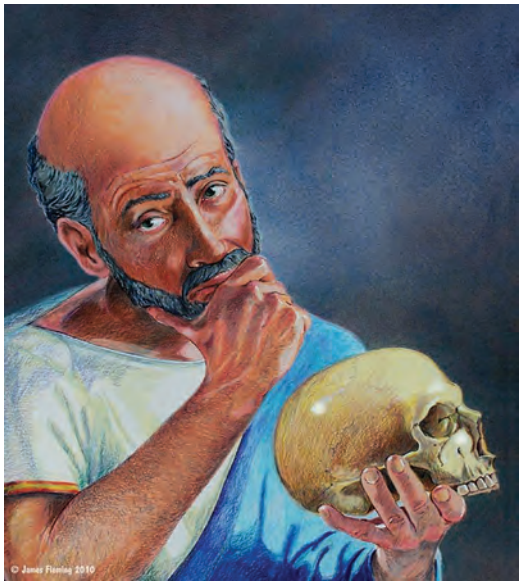

Figure 1. Hippocrates studding the anatomy of a human skull, aquarelle by James Fleming, 2010.

is displaced out of the glenoid fossa, is due to either imbalance in the neuromuscular function, or structural deficit. Conservative interventional methods in its management include symptomatic pain relief with analgesics, and manual reduction (4). This simple approach was in fact introduced about 2500 years ago by Hippocrates and his pupils (2).

We report, as an example, an 80 year old female patient with a missed case of bilateral MD, treated conservatively with the use of the Hippocratic method. Our case may present nothing new, but it links some misunderstood facts and contributors with the truth of the ancient Greek Corpus Hippocraticum. We furthermore propose the term "mandibular dislocation" for such a condition, as a more accurate medical definition in term of clinical anatomy.

A modern case report

The patient was admitted to our private department presenting with an open bite, an anxious face and mild drooling of saliva. Physical examination, after palpation of the

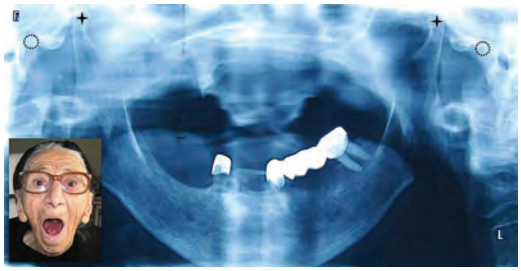

Figure 2. Clinical appearance of a bilateral dislocation of the mandible, an 80 year old female patient and x-ray depicting the bilateral dislocation of the mandible (circles=the articular positions, stars=the dislocated condyles).

preauricular region, revealed emptiness in the joint space. The patient remembered a "strange feeling of movement in her mandible after yawning" 6 months prior to her visit. Her scoring with the validation scale tool "Greek Brief Pain Inventory" (GBPI) was 3 (0=no pain, 10=severe pain, plus optical scale), reporting almost no pain at all. Her nutrition included mostly ground food and soups because her oral cavity presented an almost complete absence of teeth. Furthermore, she had refused to use dentures. Supplementary pathology included osteoporosis, diffused athropathia, mild anaemia, and vascular dementia (MMSE=17). X-ray revealed a bilateral MD of Type III by Akinbami's classification (the head of the condyle is high-up in front of the base of the eminence) (Figure 2) (4-5). Manual reduction of the MD under general anaesthesia and muscular relaxants was selected to avoid a possibly severe and painful incident. There were no immediate complications and the patient was discharged with the suggestion of follow-up by a maxillofacial surgeon.

Discussion

In Corpus Hippocraticum (cca 5th-4th century BC), in the treatise "Mochlicon" there is a very accurate description of MD, attributed to tendon relaxation and muscle atrophy,

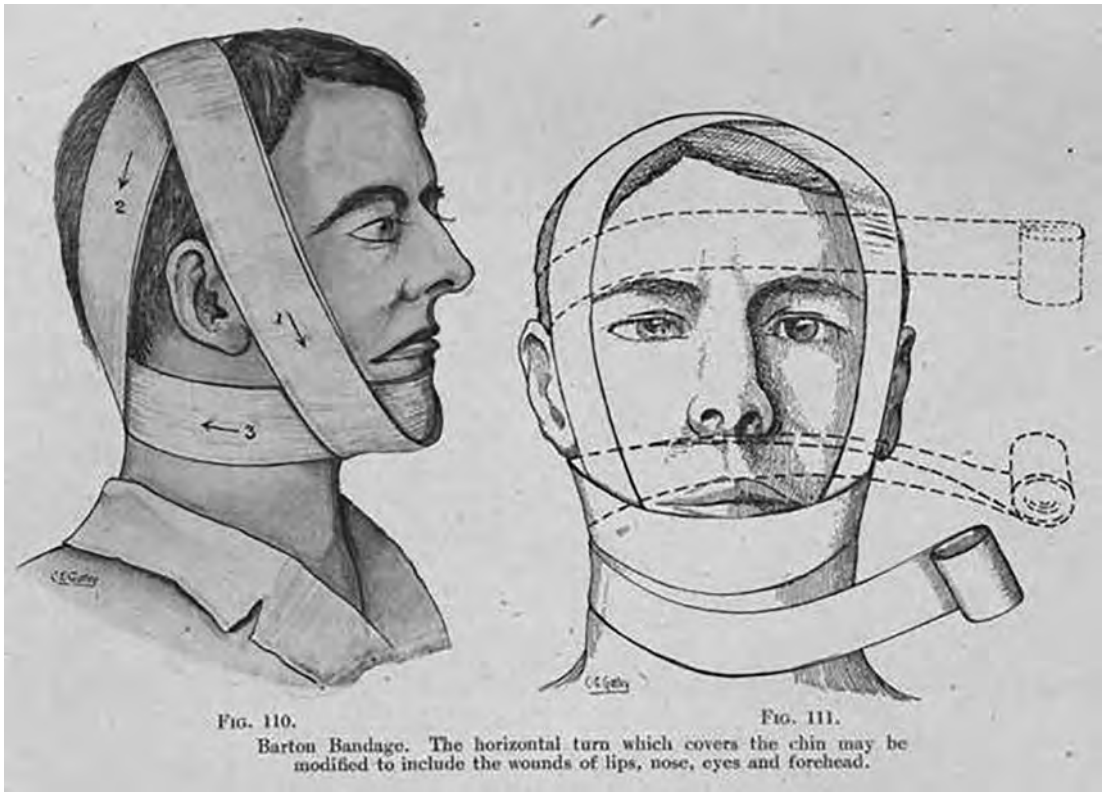

Figure 3. Gorley CE. The Barton Bandage, Centre for the History of Medicine: https:/collections.countway.harvard.edu/onview/items/show/17962.

classified as unilateral and bilateral, mainly caused by yawning. It was described as a rare condition, which needed to be quickly addressed, while the bilateral form was noted as more harmful. The treatment was also thoroughly described: "The patient is put in a lying or sitting position, while an assistant must hold the head tightly in a steady position. The physician grabs the mandible with his two arms from inside and outside the oral cavity (today: the external oblique line and the area under the mandible), from both sides, left and right, performing 3 manipulations simultaneously. He lifts up the mandible, pushes it backwards while closing the oral cavity, all at once. Painkillers should be given (today opioids). The mandible should be fixed in its normal position with the aid of bandages (today: Barton bandage)" (2). This method is still in use. Although Hippocrates was the one who introduced the practice of bandages for the mandible to be fixed, the international nomenclature attributed it to naval surgeon, William Paul Crillon Barton (1786-1856) (Figure 3).

Furthermore, researchers believe that Lewis modified the procedure in 1981 by proposing the sitting position (4-6), while this had already been suggested in the Hippocratic era (Greek fragment: καθημένου του ανθρώπου) (2). The inveteracy of this method may be further certified by both the reference and the illustration in the manual of Appolonius of Citium in Cyprus (c. 1st century BC) (Figure 4) (7).

Apart from the Hippocratic understanding of the role of the weakening of the local musculature, the "open lock sign" was also known (Greek fragment: προέχει η κάτω γνάθος ... ξυμβάλλειν ου δύναται). The original ancient text describes what surgeons nowadays express as "the mandible

Figure 4. Apollonios von Kition. De articulis. Buchmalerei nach dem Vorbild einer Zeichnung in einer antiken Handschrift Einrenkung des Unterkiefers. Florenz Biblioteca Medicea Laurenziana Plut 74 7 fol 198v. (Book illustration from the example of a drawing in an ancient manuscript of reduction of the lower jaw).

is postured forwards…an open bite". Furthermore, medico-philosophers of the Hippocratic era, faithful to the ancient Greek tradition of accurate anatomic orientation, described exactly the MD as "the mandible leaving its place". Thus, a bone is moved from its normal articular position, not the joint itself (2). Having in mind that a joint cannot be moved, we may note that the expression used by a plethora of modern researchers "temporomandibular joint dislocation" (3-5, 8) is in fact an incorrect anatomic term which should be abandoned.

Our patient reported no pain, while an acute dislocation is usually a very painful clinical entity (4). Her age and nutrition (muscular atrophy), combined with vascular dementia may explain her condition. Furthermore, her low cognitive reserve, her rural residency, the complete absence of teeth and dementia all contributed to a neglected and/or non-perceptible incidence. In the end, she was treated with a method dating back to Greek antiquity, which is about 2500 years old. The bilateral MD dislocation was easily reduced using the Hippocratic bimanual intraoral technique under sedation, with two medical personnel present (2, 9). Pain, during and after the procedure, in our case was dealt with the administration of opioids (codeine and tramadol). In the Hippocratic era, herbal drugs based on meconium (Greek: μηκώνιον), most probably the Papaver Somniferum plant, were considered as narcotics and painkillers (10). Moreover, Thessaly's endemic plant, mandrake (known also as mandragoras, Greek: μανδραγόρας) was also widely administered as a sedative and narcotic drug during surgical procedures (11). Personalized confrontation of the pain with opioids, represents one more similarity to be added to the apposition of ancient Greek and modern maxillofacial surgery.

Although various interventional Hippocratic techniques remain timeless, the management of facial trauma in the Hippocratic era is underestimated and usually neglected when historians trace the origin of modern methodology. Techniques from the past were named after modern physicians who were considered to be the innovators, whilst the true pioneers remain unappreciated. The advancement of medicine has only further established the Hippocratic dogma for the reduction of MD (12).

Epilogue

The Hippocratic School of Medicine, through thorough observation and extended acquired experience, succeeded in establishing surgical techniques which have endured time. The Hippocratic intervention

for reduction of a MD is a simple non-surgical procedure still used by modern maxillofacial surgeons. Due to its global acceptance, one may even say that it is a medical dogma, a timeless manoeuvre which, in most situations, is the right conventional intervention to be used in MD.

Authors' contributions: Conception and design: VT and GT; Acquisition, analysis and interpretation of data: VT; Drafting the article: GT; Revising it critically for important intellectual content: VT and AF; Approved final version of the manuscript: AF.

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*Data for this survey were collected from PubMed/MEDLINE using the keywords Bosnia and Herzegovina and 2017. The search was performed on the 21st of May 2018.

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by Nerma Tanović

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