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Ismet Mujezinović (1907-1984), "Landscape in Živinice", 1927, oil on canvas, 550x700 mm. Courtesy of the International gallery of portrait Tuzla.

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Editorial

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Who monitors the mentors?

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Key words: Mentoring ▪ Monitoring ▪ Metrics ▪ Medicine.

Who are mentors? What are their roles?

In a global sense it is not difficult to agree that anyone wo contributes to the growth and development be it somatic, intellectual, educational or any other form is a mentor. There is no argument that the parents, teachers, coaches, special skill instructors and other similarly engaged individuals can collectively be viewed as mentors They probably instill more important formative traits than any designated mentors, in an individual's mature life. No intention to praise or criticize the name selection for these early mentors, even though other authors may find this a critical distinction (1). There are several recent articles, elaborating on various levels and/or environments of the formal mentoring processes in science, particularly in biomedical arena (2), similarly (3) offering a novel model of mentoring in biomedical sciences, arriving to concepts of viewing certain metrics, as well as the environmental dynamics of the mentoring process. It is worth mentioning that the current developmental sociologists view the role of family, namely the early life mentoring by the parents and grandparents, as having a critically important value.

Thus for the purpose of this editorial I will define mentors as individuals who prepare those selected or assigned to them to grow, get better and ultimately excel above and beyond their mentors' knowledge, skills and ability in a particular area or field. A failure of existence of such a system may be paramount to no progress, perhaps stagnation and potentially even regression in a particular field. In fact, this writer is on a record for stating that a lack of mentors, specifically in the field of orthodontics, inevitably would lead to a crisis in orthodontic education and leadership (4).

In my many years of teaching and acting as an educational administrator, I have never met, or even heard of a person whose job title was "mentor". Instead, the various mentoring functions are typically taken by, or given to the "chiefs" – Department Heads, Directors, Deans and similar administratively titled individuals. In some, but not many, job descriptions of these chiefs the task of mentoring members of their team is listed, however the specific tasks of doing this routinely and in a given time frame, are not included. If this task was given, it would be logical to also find the specific criteria how successes of performing in this assign-

ment are measured. All to me available documents are sadly lacking any such metrics.

If we reflect on our own professional development, recalling who our memorable mentors were, it is likely that we would come up with a relatively modest person, who managed to help us "see our way better". Probably it would not be a world-wide recognized or global expert, but the person who had the time and interest in us, whom we consulted when we didn't know the questions. Even if you achieved a high degree of success in your own field, chances are that your mentor remained in the background, named on your thesis or a significant paper's acknowledgments page.

Conversely, it is a bit difficult to know what one may expect to gain from their mentor, because the rules are seldom made known to either party (1). By now the reader may have perceived a hint that the entire process of mentoring, in order to move in a positive direction, must have some metrics or measures for evaluating the mentoring effectiveness developed and accepted. We should be prepared to value mentoring activities with the same yardstick that we measure the number and importance of our published work, the numeric value of our research grants, frequency of our invited lectures and awards given for our scientific opus. Best to my knowledge, such equalizers have not been developed. It is the high time to do so!

In an institution where I was appointed in my early academic career as a faculty, a modest and entirely voluntary student mentoring program was in place. At the start of their freshman year, during the orientation meeting, the students were asked if they were interested in being assigned to a particular faculty, who would act as their academic mentors. I "took" 16 students, offered to see them on the basis of the open door policy. I also scheduled a meeting, where we would introduce ourselves to each other. Of my 16 mentees 4 came to the meeting, 2 more responded to my follow up mail, I stayed in touch for 3 years with one student, the one who was interested in applying for a program that I was directing. An interesting part of the story is that when the supposedly participating students were asked, at the end of the year, to fill a form, giving feedback evaluating their individual experience with the program, as well as the effectiveness of their mentor, all 16 responded. They all stated that I was an excellent mentor and that I helped them throughout the year. Again, this included the students that I have not seen that entire year!

One question asked what was good and attractive, and also what was not good about the existing program. The most common answer was the knowledge that the mentor was "there" and could be reached within a reasonably short time, when needed.

No comments are needed.

An experiment with reality

During the periodic evaluations of administrators, one of the common remarks was that the majority of them have not actively worked on developing their successors. It was not too difficult to equate this observation with the lack of proper mentoring. In the area where this author spent working during his last 20 plus years, prior to his retirement [New York City area], there are 12 academic institutions, colleges or universities, offering medical and/or dental programs, leading to doctoral degrees.

In early 2000s, these schools employed several thousand faculty, more than 1000 employed full time. We estimated that at least 5% of these, or somewhere between 50 and 100 faculty were in a "chief"s" position. These are assumed to have had not only the leading administrative roles, but were also the prime source of academic mentoring.

During a popular and always well attended local meeting, an informal agreement was made for the "chiefs" who had any written mentoring guidelines or protocols, to share them with interested colleagues. We at New York University Medical Center, comprising of the College of Medicine and the College of Dentistry and Nursing, accepted the task of collecting, tabulating and then distributing the fruits of this somewhat naïve idea. We expected and were prepared to process several hundred of documents. Only 26 were received, more than half of these from our two NYU colleges. Not totally discouraged by this painful lack of interest, we carefully read and processed the few we received.

The summary was sent to all who contributed and it was informally made known that essentially any of the non-contributors could obtain the document, but on the basis of *nomina odiosa*. So what was contained in the received documents? First and perhaps the most relevant – mentoring of young faculty on their way to becoming future mentors is not a high priority of the chiefs! Most of the ones who report some form of formal mentoring did so because they received specific directives from the recommendations of the accrediting bodies and not from their administrative supervisors [typically the Deans]. One other interesting and repeating situation described a formal mentoring of a young, promising faculty, groomed to become the Departmental Head. Alas, an offer from the private practice sector, bearing the double income, prevailed and took that targeted for academic greatness individual in a different direction. The moral of that story is that it is difficult to ignore the cost of mentoring the young faculty, particularly when the outcome is negative, not because the process was inadequate, but because attractions of larger pay is nearly impossible to out-duel, even when both the protagonists and the process excel.

Could we agree on how the mentor's effectiveness is measured?

This is, admittedly, the hard part. I suppose that if it were easy, someone would have, by now, developed sensible metrics. Why is this not an easy task? It is probably fair to state that no two mentees require the same form or degree of mentoring. This, however, can be said for the task and obligation of writing and publishing. This is true for many other forms or aspects of our functionality. One not too difficult measure to implement is the feedback or evaluation of those who were being mentored. Of course this would be a very subjective process, easily seen as retaliatory. Let us not forget that any judging or comparing performances, be it in sports, theatrical arts and certainly in sciences is already subjected to measuring and judging through our personally biased views.

The continuous scales or axes have been developed by statisticians and sociologists, where they manage to "measure" extremely subjective or personal sensations, for instance pain, anxiety, sorrow, happiness and others (5). Could we project that similar methods could be developed for the subjective measuring of the mentoring process and its effectiveness? Next powerful measure would be following the mentor generated plan of action. At some point in time, call it the T1, a plan, containing the final outcome prediction, but also the intermittent, say 6 months or one year intervals, namely T2, then T3 and so on to Tn. It would seem that the temporal element must be added as a crucial part of this evaluation. Are there any predictable problems with this? Of course! Who will hang the proverbial bell to the cat's neck? That is to say, who will look at such a 'contract' and say: NO, this is not enough? Or conversely: you are aiming too high, this is unrealistic!

Controlling the mentors (It is a hard work, but someone must do it!)

Early on in my career I was asked these questions: Do you know what mentoring is? Do you think you can be an effective mentor? How does one answer that? Surely I believed that I could and I would. It occurred to me, however, to ask how I would know if I was doing a good job of mentoring my staff and my students. The metrics needed to determine my effectiveness of mentoring were not presented.

Equally, or even more challenging was when, two decades later, I was appointed to a administrative committee, changed with evaluating the mentoring effectiveness of my peers, Deans, Department Heads and Program Directors. No working rules were given and it was clearly stated that we, the committee members, were expected to decide how to evaluate the mentoring skills and effectiveness, fully aware of the possibility that those who we evaluated today may be evaluating us tomorrow. We asked the respective chiefs to submit a brief written narrative of their mentoring actions. Submitted responses included "regular meetings to discuss the future plans", "some success in academic rank promotion and tenure", "taking individual member out for a meal, a play or a concert, during which time the member's plans were discussed". Tricky and perhaps even risky practice clearly not recommended as a routine tool of mentoring. However, the short of it was that we have not discovered any existing tools, nor did we create a "white paper" containing the easy to understand and apply metrics. Conversely, we have submitted a list of the basic parameters for the evaluation of mentoring actions (6).

It was identified that:

1. In order to move forward, the mentoring process must exist;
2. Define the roles of chiefs and mentors;
3. Where needed, separate the two roles;
4. Introduce the time dimension, set it along with the quality and quantity;
5. Provide the mechanism for the negative evaluation; be prepared and willing to deal with it!

What are or can be incentives to be a mentor?

Even though one could expect substantial territorial (e.g. location of the school or institution) and socio-economic differences, I suspect that incomes and other material incentives for mentors are quite similar. Perhaps this needs to be restated by saying that those who devote a significant part of their job to mentoring, typically don't get wealthy from it. In the American society the somewhat sarcastic expression that mentoring is not a billable procedure, fairly depicts the choices that the top people, the high ranking experts in their field elect to provide the billable services, such as patient care, contact hours of teaching, guest lecturing and similar.

There must be some other, non-tangible benefits to mentoring. One that readily comes to mind is the opportunity to travel. This is either because of the mentor's own scientific reputation and name recognition, or thanks to invitations of those who we monitored during their growing phases.

It is also true that the mentors are often invited to serve on the thesis committees, which is in many places separately paid. Therefore the research and thesis committee membership may supplement mentors incomes, some time by a sizable margin. [ref.: Personal communication with numerous Deans in many countries on several continents]. As a person with clinical specialty training, who also served as a mentor to literally hundreds of students and young faculty, I can personally vouch that it is easier to make a comfortable living by seeing patients, rather than advising my mentees.

Concluding remarks

At the end of this exercise the reader is justified to ask what I really think of and how strongly I support the academic mentoring. I'll try to make myself clear. Most of discussion in this issue concerns the field of medicine and its related scientific basis. I'm assuming that my own thought coming largely from the dental field, will help enlarge the playing field, while clearly showing that the grass is of the same color and quality. I will further assume that the situation is not all that different in other scientific areas. The prevailing conditions and practices are too similar to believe that things are different.

Allow me to take a stab in a diametrically opposite direction. Suppose that a strict governmental decree is announced, entirely prohibiting any form of mentoring. Who would be affected the most? Whose livelihood would be disturbed the most?

I certainly don't claim to be a deciding arbiter, but if I had to, this is how I would answer. First, the Earth would not stop from spinning, Saturday will still follow Friday. In other words, the world could survive such a ruling. I dare guess that in most of the biomedical fields mentors would readily and easily refocus on other functions, with which they are more than familiar. So these "former mentors" would surely survive. What then happens when many of the current mentors age, retire and die?

I envision an alternate system to emerge, one whose parameters have not been yet developed. This, however, brings me to the verses of the brilliant Croatian poet and writer, Miroslav Krleža: "Never is such that it isn't somehow, and never will be that it won't be somehow".

Many of the suggested tenets should be formally accepted and put to task. The optimist in me is screaming that, if the Greeks left their legacy defining the terminology of actions and processes that we engage in, including the definition of the mentorship, perhaps the future biomedical scientists will come up with ways to develop their successors. It is unfortunate that we cannot consult with Krleža as to how such things will happen.

Conflict of interest: The author declares that he has no conflict of interest.

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Original article

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Trends of inflammatory markers and cytokines after one month of phototherapy in patients with rheumatoid arthritis

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Key words: Interleukin ▪ Phototherapy ▪ Rheumatoid arthritis ▪ Tumor necrosis factor-α.

Objective. to evaluate changes in the expression of tumor necrosis factor-α in patients with rheumatoid arthritis submitted to phototherapy. **Materials and methods.** This was an open label study, enrolling ten patients. The phototherapy scheme within a range of 425 to 650 nm, 11.33 Joules/cm^2, 30 cm above the chest was as follows: a) 45-min daily sessions from Monday to Friday for 2 to 3 months; b) three, 45-min weekly sessions for 1 to 2 months; c) twice weekly 45-min sessions for 1 to 2 months, and d) one weekly session for 1 to 2 months until completion. Erythrocyte sedimentation rate, C-reactive protein and rheumatoid factor were measured in peripheral blood and tumor necrosis factor-α, interleukin-1β, and interleukin-10 in leukocytes by quantitative real-time Reverse transcriptase-Polymerase chain reaction. In all the patients the next indexes: Karnofsky scale, Rheumatoid Arthritis-specific quality of life instrument, Steinbrocker Functional Capacity Rating and the Visual Analog Scale were evaluated. **Results.** Erythrocyte sedimentation rate, C-reactive protein, and rheumatoid factor declined notoriously after the indicated sessions. In gene expression, there was a tendency in tumor necrosis factor-α to decrease after 1 month, from 24.5±11.4 to 18±9.2 relative units, without reaching a significant statistical difference. The four tested indexes showed improvement. **Conclusion.** Phototherapy appears to be a plausible complementary option to reduce the inflammatory component in rheumatoid arthritis.

Introduction

Rheumatoid arthritis (RA) is a systemic inflammatory disease characterized by joint pain, swelling, stiffness, and progressive destruction of the small joints. Patients with RA should be treated appropriately in order to improve symptoms and inhibit structural joint damage. Treatment of RA has improved over the past decade. Of the treatment options available, Non-steroidal anti-inflammatory drugs (NSAID) are the most widely used agents for symptomatic treatment. However, these drugs have several adverse effects (1). More options are low-dose glucocorticoids which have a modifying effect on structural damage in early RA (2) and disease-modifying antirheumatic drugs (DMARDs) such as hydroxychloroquine, methotrexate (MTX), leflunomide, cyclosporine, sulfasalazine, azathioprine, cyclophosphamide and biologics (3, 4). To

date, anti-Tumor necrosis factor-α (TNF-α) agents represent a milestone in RA treatment (5-7).

Although new therapies are increasingly available (8), a significant unmet medical need continues for patients with RA who have had inadequate response to prior treatments and require safe and effective therapy using a different mechanism of action. Alternative and complementary treatments for RA have been explored in many countries. One of these alternatives is phototherapy with diverse light spectra including Ultraviolet (UV), laser Photodynamic therapy (PDT), Light emitting diodes (LED) etc. (9, 10).

Our main objective was to evaluate the changes in the expression of TNF-α, Interleukin (IL)-1β and IL-10 in patients with RA while they are being submitted to a complementary treatment with phototherapy.

Methods

Study population

This was an open label study to evaluate the effect of phototherapy in patients with RA who met the criteria established by the American College of Rheumatology (ACR) (11). We included consecutive patients attending the Research Department, of the Maternal-Perinatal Hospital "Mónica Pretelini Sáenz" (HMPMPS), Health Institute of the State of Mexico (ISEM), Toluca, Mexico, in the period 2010-2012. Those with fewer than four criteria of the ACR, previous fractures, chronic diseases that limit functional capacity, other arthropathies, overlap syndromes, and no agreement of the patient to participate in the study were excluded. Patients who failed to comply with the phototherapy treatment program or severe disease progression were discarded. There was no any kind of restriction to the prescribed pharmacotherapy.

Phototherapy

With the patient in supine position, after registering vital signs (blood pressure, heart rate, respiratory rate, and temperature), weight, height, and capillary glucose determination, we proceeded to place the phototherapy lamp (Federal Ministry of Health registration number: 1694E95) within a range between 425 and 650 nm, 11.33 Joules/cm^2, 30 cm above the chest. The decision of placing the lamp above the chest was based on the location of the great vessels and the thymus as the aim of the treatment was to get a systemic effect rather than a local one.

The phototherapy scheme was the following: a) 45-min daily sessions from Monday to Friday for 2 to 3 months; b) three 45-min sessions per week for 1 to 2 months; c) twice weekly 45-min sessions for 1 to 2 months, and d) one weekly session for 1 to 2 months until completion. Weekly frequency and progressive reduction of phototherapy sessions were determined according to the patients' own improvement.

Clinical follow-up

The patients' data was obtained from their medical history. Clinical evaluation was performed by the Research Team Leader once weekly, including the next indexes: Karnofsky scale, Rheumatoid Arthritis-specific quality of life (RAQoL) instrument, Steinbrocker Functional Capacity Rating and the Visual Analog Scale (VAS). An essential aspect of the study was absolute respect for the management, evaluation, and subsequent appointments instituted by the treating Rheumatologist. Phototherapy alone was considered for naïve patients with contraindications for the antirheumatic drugs.

Biochemical assessment

Laboratory tests were done in the first clinical visit and 4 weeks after the initiation of

treatment. Fasting blood samples (10 ml) were taken at the HMPMPS Laboratory at an early-morning after an overnight fast. Serum samples were analyzed for globulin (Dimension Rx L Max, Dade Behring, USA), hemogram (Advia 120, Bayer Health, USA), Erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), and Rheumatoid factor (RF). All these tests were measured according to standardized procedures recommended by the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC).

Leukocyte collection and storage

Blood samples were taken by venipuncture in tubes (Vacutainer) containing Ethylenediaminetetraacetic acid (EDTA) as anticoagulant for subsequent centrifugation at 2,500 rpm for 10 min. Using a 1-ml micropipette, leukocytes were separated and deposited in sterile Eppendorf® 1.5-ml tubes. Once separated, the leukocytes were purified in 1 ml Red blood cell (RBC) ACK Lysing buffer (ACK), stirring by gentle inversion and allowing these to sit for 1 min at 30°C. Following this, they were centrifuged at 2,000 rpm for 5 min, the supernatant was discarded, and 1 ml of ACK was again added, repeating the process until visualizing a pellet without RBC. The final step was the addition of 100 µl of phosphate buffer and resuspension for further storage at -80°C (Forma -86ºC ULT Freezer, Thermo Electron Corporation, USA) until analysis, which was carried out in the Laboratory of Molecular Biology, Medical Sciences Research Center (CICMED), Autonomous University of the State of Mexico (UAEMex).

Gene expression

Messenger RNA (mRNA) was isolated using the Magna Pure LC RNA Isolation Kit III and retrotranscribed with the Transcriptor High Fidelity cDNA Synthesis Kit (Roche) to obtain complementary DNA (cDNA). Samples were then quantified using a nanophotometer set (Nano Photometer, Implen) at two wavelengths (260 nm and 280 nm), with an acceptable degree of purity between 1.8 and 2.

Quantitative real-time Reverse Transcriptase-Polymerase Chain Reaction (qRT-PCR) was performed in a 7500 Fast Real Time PCR System (Applied Biosystems, Applera UK, Cheshire, UK), using the TaqMan® Gene Expression Assays (Life Technologies, USA) for TNF-α (Catalog #4331182), IL-1β (Catalog #4331182), and IL-10 (Catalog #4331182), following the manufacturer's instructions. The relative expression of these genes was calculated through the $2^{-\Delta\Delta CT}$ method against 18S (NCBI: NC_000012.11) as follows: fw: 5'-ctttggtatcgtggaaggactc-3', and rv: 5'-gtagaggcagggatgatgttct-3' (Catalog #Hs99999901-s1; Life Technologies).

Ethics statement

The protocol was approved by the Research Committee of the HMPMPS (November 2010) and followed the Declaration of Helsinki indications. All patients were asked to sign written informed consent.

Statistical analysis

Descriptive analysis, Wilcoxon test to compare whether the group presented differences through time and Spearman correlation were performed with the SPSSP v. 17 program. A difference was considered significant at $p \leq 0.05$. Results are expressed as absolute numbers, means ± standard deviation (SD) for all variables except TNF-α, IL-1β and IL-10 that are expressed as absolute numbers, means ± standard error (SE).

Results

Anthropometric data

A total of 10 women, mean age of 41.2±8.8 years, with RA were enrolled for this study. Mean time within the protocol was 140±7.7 days. The average number of antirheumatic drugs that patients were taking at the time of the initiation of the protocol was of 3.7 (Table 1).

Clinical evolution

Table 2 shows the results of the evaluated scales. While the Karnofsky increased 1.3 times, the RaQol questionnaire was reduced by almost half. Additionally, the Steinbrocker Functional Capacity Rating improved from Class III to Class II. Finally, the VAS showed a reduction in pain from "dreadful" to "annoying".

Laboratory analysis

There were no differences in the hematological evaluation. The acute inflammation variables declined notoriously after the indicated sessions. In gene expression, there was a tendency in TNF-α to be decreased after 1 month, from 24.5±11.4 to 18±9.2 relative units without reaching significant statistical difference. Neither IL-1β nor IL-10 showed significant statistical differences (Table 2).

IL-1 and IL-10 showed a significant negative correlation in their trends (-0.829, p=0.042). On the contrary, there was a positive correlation in the reduction values of TNF and the VAS (0.894, p=0.041).

The Karnofsky scale showed a negative correlation with the RaQol (-0.851, p=0.002) and VAS (-0.757, p=0.011) scales. The last scale showed a positive correlation with RaQol (0.665, p=0.036), Steinbrocker Functional Capacity Rating (0.774, p=0.009) and with the TNF-α reduction (0.894, p=0.041).

Table 1 General characteristics of the population

Case	Age*	Disease duration†	Concomitant treatment
1	41	4	Metamizol 500 mg/day PO‡; Paracetamol 650 mg/day PO; Ketorolac 30 mg/day IM‡
2	47	60	Methotrexate 10 mg/week PO; Hydroxychloroquine 200 mg/day PO; Acemetacin 60 mg/12 h PO; Methylprednisolone 40 mg bimonthly IM; Diclofenac 75-150 mg/day PO
3	32	58	Methotrexate 15 mg/week PO; Sulfasalazine 500 mg/12 h PO; Diclofenac 100 mg/12 h PO
4	41	9	Prednisone 10 mg/day PO; Diclofenac 100 mg/12 h PO; Paracetamol 500 mg/12 h PO; Meloxicam 15 mg IM only 2 doses
5	44	59	Methotrexate 5 mg/week PO; Deflazacort 2 mg/day PO; Diclofenac 100 mg/12 h PO
6	44	45	Methotrexate 15 mg/week PO; Sulfasalazine 500 mg/8 h PO; Prednisone 20 mg/day, 10 days/month PO; Acemetacin 60 mg/12 h PO; Fluoxetine 20 mg/day PO
7	48	34	Leflunomide 20 mg/day PO; Sulfasalazine 500 mg/12 h PO; Diclofenac 100 mg/12 h PO
8	42	2	Leflunomide 20 mg/day PO; Hydroxychloroquine 200 mg/day PO; Deflazacort 3 mg/day PO; Naproxen 275 mg/12 h PO; Paracetamol 300 mg/12 h PO
9	21	17	Prednisone 75 mg/day P.O. §Dexamethasone 8 mg IM only 4 doses; §Ibuprofen 400 mg/12 h PO
10	52	120	Chloroquine 150 mg/12 h PO; Sulfasalazine 2.5 g/day PO; Diclofenac 100 mg/day PO

*Years; †Months; PO=oral administration; IM=intramuscular; ‡Self-medication, §This treatment was suspended 7 months previous to the study due to pregnancy.

Table 2 Clinical and laboratorial characteristics

Characteristics	Values of laboratory tests		p value
	Basal	After‡	
Hb (g/dl)*	12.4±1.6	12.7±1.6	0.134
Ht (volume %)*	38.6±4	39.35±4.9	0.386
Leukocytes (cells/mm3)*	6390±1757	6060±1509	0.327
Granulocytes (cells/mm3)*	4554±1561	4302±1414	0.285
Lymphocytes (cells/mm3)*	1360±536	1270±309	0.386
Monocytes (cells/mm3)*	284±80	296±88	0.203
CRP (titers)*	1:204 ±169	1:122 (±186)	0.008
ESR (mm/h)*	45±14	38.6±13	0.017
Rheumatoid factor (titers)*	1:808 ±1554	1:178 (±387)	0.026
IL-1β (RU)†	0.23±0.18	0.66±0.32	0.917
IL-10 (RU)†	1.6±1.2	0.58±0.32	0.374
TNF-α (RU)†	24.5±27.9	18±22.6	0.249
Karnofsky scale (points)*	46±17.1	64±12.6	0.005
RAQoL (points)*	25.7±8.5	13.9±6.1	0.001
VAS (points)*	6.9±2.4	3.2±2.2	0.001

*Mean±SD; †Mean±SE; ‡One month of phototherapy; CRP=C reactive protein; ESR=Erythrocyte sedimentation rate; Hb=Haemoglobin; Ht=Haematocrit; IL=Interleukin; RAQoL=Rheumatoid Arthritis-specific Quality of Life; RU=Relative units; TNF-α=Tumor necrosis factor-α; VAS=Visual Analogue Scale.

Discussion

DMARDs, TNF inhibitor treatments, or their combination are considered first-line treatment (12) in RA, but collateral effects such as hepatotoxicity and nephrotoxicity are commonly associated with the former option. In addition, immunosuppression predisposes to increased risk of infection, the potential for development of certain types of malignancy, as well as the significant increased cost of therapy.

It has been demonstrated that UV radiation poses an immunosuppression effect due in part to changes in the expression of IL-10, transformation of urocanic acid from its trans to cis isomer, and induction of CD4+ CD25+ T regulatory cells (9). Evidence indicates that phototherapy exerts a significant impact on neutrophils, the effect of which varies according to the specific type of phototherapy (13).

A previous study by Goats et al. tested the therapeutic effects of combined low-intensity laser and phototherapy upon the articular, systemic, and functional sequelae of RA affecting weight-bearing joints without finding significant differences between the active or placebo cohorts (14). To date, the majority of trials have unsuccessfully tested laser therapy with a wide range of parameters in Joules and wavelength (15). Contrariwise, the present study noted a tendency of TNF decrement.

In mice models, LED irradiation has been effective for inhibition of the inflammatory reactions caused by RA within a period of four weeks (16); but to the best of our knowledge, the present study is the first specifically designed to investigate the short-term effect of visible light and changes in peripheral TNF-α, IL-1β, and IL-10 expression. Despite the lack of significant differences after the month of follow up, we

did find significant correlation between the clinical improvement and the reduction in TNF-α.

The primary cellular effects of phototherapy are related with the interaction of photons and the intracellular molecules that absorb them, i.e., the cytochromes. Visible light is absorbed by cytochromes, many of which are located in the mitochondria. It has also been postulated that light can act as a catalyst, influencing molecules, organelles, and cells without being absorbed (17). Whether or not the primary effects of light induce the changes in inflammatory variables is a matter that has yet to be discerned. In an initial attempt, this study shows a possible reduction in TNF-α secondary to the phototherapy sessions, but more studies are needed to clarify this issue.

A limitation of this study is the low number of participants. Notwithstanding, the quantification of gene expression of inflammatory markers along with the clinical scale evaluation, gives a good support for a possible recommendation of phototherapy in RA. Another limitation of this study in terms of the interest for the clinician is that we didn't collect any activity index of the disease such as DAS28 or SDAI which are the topic of a new project.

Conclusion

Phototherapy could be useful to treat patients with RA, which is clearly evident with the reduction in the following inflammatory markers: ESR, CRP, and RF. Due to scarce knowledge on the mechanism of action of phototherapy, the results should be treated with caution.

What is already known on this subject

Rheumatoid arthritis (RA) is a chronic and painful inflammatory disease. To date, anti-Tumor necrosis factor-α agents represent a milestone of RA treatment in addition to non-steroidal anti-inflammatory drugs (NSAID), low-dose glucocorticoids, and Methotrexate. Despite the development of new pharmacological therapies, alternative and complementary treatments for RA have been explored extensively.

What this study adds

*As a complementary option, phototherapy with diverse light spectrums has shown potential clinical utility in treating RA. In this line, we evaluated the effect on inflammatory markers in RA after 1 month of phototherapy within a range of 425 to 650 nm, 11.33 Joules/cm², yielding a reduction in the levels of E*rythrocyte sedimentation rate*, C-reactive protein, and rheumatoid factor.*

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Pre-hospital use of inhaled corticosteroids and inhaled beta agonists and incidence of ARDS: A population-based study

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Objective. Inhaled corticosteroids and inhaled beta agonists were shown to decrease the lung injury in animal models. We investigated the association of pre-hospital use of inhaled corticosteroids and inhaled beta agonists with the incidence of Acute Respiratory Distress Syndrome (ARDS) in a population based cohort of hospitalized patients. **Material and methods**. Retrospective cohort study of adult patients from Olmsted County, Minnesota admitted to the hospital with at least one predisposing condition for ARDS from 2001-2008. The association with pre-hospital use of inhaled corticosteroids and inhaled beta agonists was evaluated using univariate and multivariate analyses. Primary outcome was ARDS and secondary outcome was hospital mortality. **Results.** Out of 2429 hospitalized adult patients with at least one risk factor for ARDS, 10.5% of those taking and 14% of those not taking inhaled corticosteroids developed ARDS (OR 0.72; 0.53-0.97; p<0.03). Inhaled beta agonists showed similar unadjusted protective effect; 9.7% of users and 14.4% of non-users developed ARDS (OR 0.64; 0.48-0.86; p=0.003). After adjusting for risk factors, comorbidities and severity of illness in the multiple logistic regression model, use of inhaled beta agonists, but not inhaled corticosteroids, remained independently associated with decreased risk of ARDS (OR 0.48; 0.31-0.72; p<0.001 versus 0.87; 0.57-1.29; p=0.49). The estimated protective effects were more pronounced among patients with pneumonia compared to those without pneumonia. **Conclusion.** Pre-hospital use of inhaled beta agonists but not inhaled corticosteroids was significantly associated with decreased incidence of ARDS among hospitalized patients at risk, once adjusted for baseline characteristics, predisposing and comorbid conditions, as well as severity of illness.

Key words: ARDS ▪ Corticosteroids ▪ Beta-agonists ▪ Pneumonia

Introduction

Acute respiratory distress syndrome (ARDS) is a heterogeneous syndrome of dysregulated inflammation, resulting from direct insults (e.g. aspiration, pneumonia, chest contusion, etc.), indirect insults (e.g. sepsis, shock, pancreatitis, etc.) or capillary-stress failure (e.g. ventilator induced lung injury, high altitude pulmonary edema, etc.) (1). It leads to respiratory failure with a mortality of up to 40% (2, 3). Besides attributable morbidity and mortality, ARDS leads to enormous increase in health care cost (4). Although ARDS definition has recently been updated (5), the management remains largely supportive without the therapeutic breakthroughs despite 30 years of intense research in the field. This lead to the recent paradigm shift where the investigative efforts are directed more towards the early

identification of patients at risk and prevention of ARDS.

Corticosteroids are potent anti-inflammatory agents, however previous studies of systemic corticosteroids were not fully supportive of their protective effect in ARDS patients (6-12). This could be at least in part due to systemic side effects (11). On the contrary, inhaled corticosteroids (ICS) with similar anti-inflammatory properties and direct delivery to the target organ are largely void of systemic adverse effects. Despite heterogeneity in timing of administration or the insult to the lungs, animal studies had shown promising results in attenuation of acute lung injury with pretreatment or concomitant treatments with ICS (13-17). In a large multicenter LIPS cohort we have previously shown that the proposed protective effect of ICS towards ARDS development in patients at risk is more pronounced in patients exposed to the direct, rather than indirect mechanisms of acute lung injury (18). Nearly 70% of patients on ICS in this cohort were taking inhaled beta agonists (IBA), as well. Beta agonists were previously shown to be able to enhance resolution of pulmonary edema and maintain stability of alveolocapillary membrane under baseline conditions (19, 20). We postulated that regular use of ICS and/or IBA could favorably modify dysregulated inflammatory response and result in lesser risk for the development of ARDS Therefore, we hypothesized that patients using ICS and/or IBA prior to hospitalization compared to those who were not using these medications have lower risk of ARDS development after the hospital admission.

In order to test this hypothesis, we studied a population-based cohort of patients at risk for ARDS from Olmsted County, MN.

Material and methods

The study was approved by Mayo Clinic's institutional review board as a minimal risk study (approval number 08-007804).

Study design

This was an observational retrospective cohort study designed to test whether the patients at risk of ARDS had smaller risk of developing ARDS if they used ICS and/or IBA prior to admission to the hospital. The cohort was first divided into ICS and IBA users and non-users. Subsequently, we divided the patients based on having admission diagnosis of pneumonia or not.

Study population

Population included adult patients from Olmsted County, MN, admitted to hospital with at least one risk factor for ARDS, from 2001 – 2008. The patients were excluded if they had ARDS already at the time of the admission (first 6 hours), if they died in the emergency room, or restricted their care to the comfort or hospice care.

Predictor variables

The ICS and IBA therapy exposure was determined through the electronic medical record with the consent of the patient or family. Any ICS or IBA medication either alone or in any combination was included. Baseline characteristics included: age, gender, race and Charlson comorbidity score. Previous diagnoses of chronic obstructive pulmonary disease (COPD) or asthma were included as well, as majority of patients on ICS and/or IBA were expected to have one of these two diagnoses. Clinical variables established as risk factors for ARDS development evident prior to the ARDS diagnosis were: pneumonia, pancreatitis, sepsis, shock, trauma, and multiple transfusions. APACHE III score was used as a severity of illness measure.

Outcome variables

The ARDS development was the primary outcome during hospitalization. Although

at the time of study conception the AECC standard criteria were used (21), we adapted the newer Berlin definition for ARDS and excluded patients who did not fulfill the PEEP requirement of at least 5 cm. The secondary outcome was hospital mortality.

Statistical analysis

We first assessed the distribution of the variables. The continuous variables were reported as medians with 1st and 3rd interquartile ranges (IQR), and categorical variables were reported as counts and proportions. We then performed univariate analyses for the primary and secondary outcomes based on the pre-hospital use of ICS and/or IBA. Subsequently, separate univariate analyses were done on patients with diagnosis of pneumonia and those without it. The statistical test used for univariate analyses was Pearson's Chi-Square test as all expected cell values were greater than 5. Following this, we performed multivariate logistic regression for primary and secondary outcomes on all patients by including the following variables: ICS and/or IBA, age, Charlson score, abovementioned risk factors for ARDS, asthma and/or COPD and APACHE III score. We evaluated interaction between ICS and IBA in the models. Finally, we applied a multivariate logistic regression model to a subgroup of patients who had pneumonia on admission. The only variable removed from this particulate model was pneumonia. The risk estimates were reported as odds ratios (OR) with 95% confidence intervals (CI). P value <0.05 was considered statistically significant. JMP 9.0 software and SAS 9.1.3 (SAS Institute Inc., Cary, NC) were used for statistical analysis.

Results

There were total of 2,429 patients who were admitted to the hospital with at least one risk factor for ARDS during the study period. Median age was 71 years (53-83) and 53% of patients were male. Other baseline characteristics are listed in Table 1. Of all patients, 564 were taking ICS and 626 were taking IBA with 354 taking both of these medications at the time of admission. There were 996 patients with the diagnosis of pneumonia and 1,433 without. Among 320 patients with ARDS, at the time of diagnosis, there were 158 (49%) with mild, 100 (31%) with moderate, and 62 (19%) with severe ARDS, respectively. Median partial pressure of arterial oxygen to fractional inspired oxygen concentration (P/F) ratios among patients taking and not taking inhaled medications were 106 (59; 197) and 95 (54; 176) for ICS and 128 (71; 198) and 95 (53; 175) for IBA, respectively.

Table 1 Demographic and clinical characteristics of the patients (n=2,429)

Age (year, median IQR)	71 (53-83)
Male sex (%)	1,295 (53)
White race (%)	2,156 (89)
Asthma and/or COPD (%)	1101 (45)
Charlson score, median (IQR)	3 (1-4)
APACHE III score, median (IQR)	52 (34-72)
Inhaled corticosteroid users (%)	564 (23)
Inhaled beta agonist users (%)	626 (26)
Inhaled corticosteroid & beta agonist users (%)	354 (15)

COPD= Chronic obstructive pulmonary disease.

Univariate analysis

Pre-hospital ICS use was significantly associated with lower risk of ARDS development; 10.5% of ICS users and 14% of non-ICS users developed ARDS during the hospitalization (OR 0.72, 0.53-0.97, p<0.03). However, when stratified by the diagnosis of pneumonia, it appeared that the protective unadjusted effect in all patients was mostly due to the effect on patients with pneumonia (OR 0.49, 0.33-0.72, p<0.001), rather

than among those without pneumonia (OR 0.93, 0.59-1.48, p=0.77). In regards to the secondary outcome - mortality, we observed similar results; pre-hospital ICS use was significantly associated with lower mortality in all patients; 15% of ICS users and 19% of non-ICS users died during the hospitalization (OR 0.73, 0.56-0.94, p=0.016). When stratified by the diagnosis of pneumonia, the protective unadjusted effect was only significant in patients with pneumonia (OR 0.50, 0.36-0.71, p<0.001), where among those without pneumonia there was no significant association with mortality (OR 0.92, 0.62-1.36, p=0.66) (Table 2).

Pre-hospital IBA use was significantly associated lower risk of ARDS development; 9.7% of IBA users and 14.4% of non-IBA users developed ARDS during the hospitalization (OR 0.64; 0.48-0.86; p=0.003). However, when stratified by the diagnosis of pneumonia, the protective unadjusted effect in all patients was more pronounced among patients with pneumonia (OR 0.50; 0.34-0.74; p<0.001), rather than among those without pneumonia (OR 0.72; 0.46-1.13; p=0.15). The similar was observed in regards to the mortality; pre-hospital IBA use was significantly associated with lower mortality in all patients; 12.1% of IBA users and 20.4% of non-IBA users died during the hospitalization (OR 0.54; 0.41-0.70; p<0.001). When stratified by the diagnosis of pneumonia, the protective unadjusted effect was more potent in patients with pneumonia (OR 0.45; 0.31-0.63; p<0.001), compared to those without pneumonia (OR 0.54; 0.35-0.82; p=0.003) (Table 2).

Multivariate analysis

The multivariate model included 2197 patients (90%) who had APACHE III score. When adjusted for pertinent potential confounders in a multivariate logistic regression analysis (Table 3), pre-hospital IBA use was independently protective of ARDS among all patients (OR 0.48, 0.31-0.72, p<0.001) but not use of ICS (OR 0.87, 0.57-1.3, p=0.49).

Table 2 Univariate analysis for the primary and secondary outcomes

Characteristics	Patients with ARDS (n=320)	Patients without ARDS (n=2109)	p-value	Dead (n=445)	Alive (n=1984)	p-value
Age (median; IQR)	64 (48-78)	72 (54-83)	<0.001	78 (65-86)	69 (50-81)	<0.001
Male (%)	181 (56.6)	1114 (52.8)	0.21	247 (55.5)	1048 (52.8)	0.3
White (%)	270 (84.4)	1886 (89.4)	0.13	373 (83.8)	1783 (90)	<0.001
Pancreatitis (%)	17 (5.3)	61 (2.9)	0.02	13 (2.9)	65 (3.3)	0.7
Pneumonia (%)	168 (52.5)	828 (39.3)	<0.001	227 (51)	769 (38.8)	<0.001
Sepsis (%)	142 (44.4)	649 (30.8)	<0.001	186 (41.8)	605 (30.5)	<0.001
Shock (%)	85 (16)	303 (9.3)	<0.001	144 (32.4)	244 (12.3)	<0.001
Asthma/COPD (%)	132 (41.2)	969 (46)	0.11	199 (44.7)	902 (45.5)	0.77
Trauma (%)	80 (25)	747 (35.4)	<0.001	70 (15.7)	757 (38.2)	<0.001
Multiple transfusions (%)	52 (16.2)	136 (6.4)	<0.001	60 (13.5)	128 (6.4)	<0.001
Charlson Score (median; IQR)	3 (2-4)	3 (1-4)	0.015	4 (2-5)	2 (1-4)	<0.001
APACHE III (median; IQR)	66 (47-91)	51 (32-69)	<0.001	77 (56-107)	49 (30-66)	<0.001
IBA (%)	61 (19.1)	565 (26.8)	0.003	76 (17.1)	550 (27.7)	<0.001
ICS (%)	59 (18.4)	505 (24)	0.03	84 (18.9)	480 (24.2)	0.01

ARDS=Acute respiratory distress syndrome; COPD=Chronic obstructive pulmonary disease; IBA=Inhaled beta agonists; ICS=Inhaled corticosteroids.

Table 3 Multivariate analysis for ARDS

Characteristics	All patients* (n=2197)		Patients with pneumonia* (n=884)		Patients without pneumonia* (n=1313)	
	OR (95% CI)	p-value	OR (95% CI)	p-value	OR (95% CI)	p-value
Age	0.97 (0.96-0.98)	<0.001	0.98 (0.97-0.99)	<0.001	0.97 (0.96-0.98)	<0.001
Pancreatitis	1.94 (0.96-3.7)	0.06	1.85 (0.54-5.93)	0.31	1.53 (0.61-3.56)	0.35
Pneumonia	2.47 (1.83-3.35)	<0.001	-	-	-	-
Sepsis	1.96 (1.45-2.65)	<0.001	1.89 (1.22-2.89)	0.004	1.32 (0.73-2.34)	0.35
Shock	1.69 (1.21-2.37)	0.002	2.67 (1.56-4.56)	<0.001	0.98 (0.56-1.67)	0.95
Asthma/COPD	1.12 (0.82-1.54)	0.45	1.06 (0.69-1.62)	0.80	1.21 (0.75-1.92)	0.43
Trauma	1.69 (1.16-2.44)	0.006	2.22 (1.26-3.8)	0.006	1.11 (0.57-2.12)	0.76
Multiple transfusion	2.49 (1.67-3.67)	<0.001	1.81 (0.92-3.43)	0.08	3.11 (1.86-5.09)	<0.001
Charlson Score	1.00 (0.95-1.06)	0.89	0.97 (0.89-1.05)	0.47	1.03 (0.94-1.11)	0.52
APACHE III Score	1.02 (1.01-1.02)	<0.001	1.01 (1-1.02)	0.005	1.025 (1.02-1.03)	<0.001
IBA	0.48 (0.31-0.72)	<0.001	0.45 (0.27-0.74)	0.001	0.49 (0.22-0.99)	0.05
ICS	0.87 (0.57-1.3)	0.51	0.86 (0.52-1.41)	0.56	0.65 (0.28-1.35)	0.26

*All patients who had APACHE III score recorded. COPD=Chronic obstructive pulmonary disease; IBA=Inhaled beta agonists; ICS=Inhaled corticosteroids.

The interaction between IBA and ICS was not statistically significant (p=0.22). This adjusted protective effect of IBA was slightly more pronounced among 884 patients with pneumonia (OR 0.45; 0.27-0.74; p<0.001), compared to 1313 that did not have pneumonia (OR 0.49; 0.22-0.99; p=0.05), however, the interaction with ICS variable in the latter subgroup was significant (p=0.016). The similar protective effect was observed for mortality as a secondary outcome (Table 4). Among all patients, IBA use but not ICS use was protective of mortality (OR 0.44; 0.30-0.65; p<0.001) versus (OR 0.86; 0.59-1.24; p=0.43), respectively. The interaction between IBA and ICS was not statistically significant (p=0.15).

Table 4 Multivariate analysis for mortality

Characteristics	All patients* (n=2197)		Patients with pneumonia* (n=884)		Patients without pneumonia* (n=1313)	
	OR (95% CI)	p-value	OR (95% CI)	p-value	OR (95% CI)	p-value
Age	1.01 (1-1.02)	<0.001	1.02 (1.01-1.03)	<0.001	1.01 (1-1.02)	0.03
Pancreatitis	0.82 (0.37-1.69)	0.60	0.98 (0.23-3.5)	0.98	0.84 (0.29-2.14)	0.72
Pneumonia	1.6 (1.19-2.15)	0.001	-	-	-	-
Sepsis	1.09 (0.82-1.44)	0.53	1.24 (0.82-1.85)	0.3	1.04 (0.62-1.76)	0.86
Shock	2.13 (1.56-2.92)	<0.001	1.92 (1.11-3.29)	0.02	2.18 (1.33-3.55)	0.002
Asthma/COPD	0.9 (0.67-1.199)	0.47	0.99 (0.68-1.47)	0.99	0.77 (0.49-1.18)	0.23
Trauma	0.76 (0.52-1.09)	0.14	0.8 (0.42-1.44)	0.47	0.83 (0.45-1.51)	0.54
Multiple transfusion	2.39 (1.6-3.54)	<0.001	2.54 (1.36-4.7)	0.004	2.29 (1.35-3.84)	0.002
Charlson Score	1.09 (1.03-1.15)	0.001	1.07 (0.99-1.15)	0.07	1.09 (1.02-1.19)	0.01
APACHE III Score	1.03 (1.02-1.03)	<0.001	1.02 (1.01-1.02)	<0.001	1.03 (1.03-1.04)	<0.001
IBA	0.42 (0.28-0.62)	<0.001	0.44 (0.27-0.69)	<0.001	0.44 (0.21-0.83)	0.01
ICS	0.88 (0.6-1.27)	0.5	0.74 (0.46-1.17)	0.2	1.13 (0.59-2.07)	0.71

*All patients who had APACHE III score recorded; COPD=Chronic obstructive pulmonary disease; IBA=Inhaled beta agonists; ICS=Inhaled corticosteroids.

Discussion

In this retrospective cohort study of adult patients from Olmsted County in Minnesota, admitted to the hospital with at least one risk factor for ARDS, the pre-hospital use of IBA was significantly associated with decreased risk of ARDS when adjusted for baseline characteristics, predisposing conditions and severity of illness. Although the overall estimated effect of ICS was in the protective range, this did not reach statistical significance. Overall, adjusted protective effects for ARDS were more pronounced in patients with pneumonia in contrast to those who did not have pneumonia. The interaction between IBA and ICS was only significant in a subgroup of patients without pneumonia. This is the first study to our knowledge that showed protective adjusted effect of IBA on development of ARDS among patients at risk.

The definite role of ICS and IBA in the prevention and early treatment of ARDS has not been established yet, despite a solid body of pre-clinical evidence (6-12). Besides the direct delivery to the target organ, ICS and IBA are void of systemic adverse effects, and this makes them the prime candidates for the lung injury prevention, especially due to direct mechanisms (epithelial injury). Recently, we demonstrated that pre-hospital use of ICS exerts stronger protective effect on patients with direct rather than indirect risk factors for ARDS where 70% of the patients on ICS were receiving IBA, as well (18). Preclinical studies of ICS delivered prior to pulmonary injury showed significantly reduced pro-inflammatory cytokines and improvements in oxygenation among animals in the intervention group (16, 17). The beta agonists have previously established its role in maintaining the stability of the barrier function under baseline conditions (20). This is very important as the early use of IBA and/or ICS in patients with relatively intact epithelium may allow the full effect of the medications, prior to the injury seen in fully established ARDS. Timely administration of inhaled medications may result in their more uniform distribution, thus allowing them to exert their protective effects. On the contrary, in fully established ARDS, inhaled medications cannot reach the target site due to heterogeneous nature of ARDS. Moreover, the affected epithelium is dysregulated and dysfunctional, which reduces the response to medications. Perhaps, this can explain while IBA failed to show the therapeutic effect in ALTA study (22). However, this is speculative and further investigative studies are needed to confirm afore mentioned hypotheses. The ongoing Lung Injury Prevention Study with Budesonide and Beta agonist, formoterol (23) is the first randomized controlled trial of ICS and IBA to prevent pulmonary dysfunction and ARDS, which may shed more lights on the topic.

We have previously studied in the LIPS cohort the role of ICS and IBA on ARDS development and showed that ICS, rather than IBA, could potentially have protective effect, especially in a subgroup of patients with at least one risk factor for the direct lung injury. It is not readily apparent why this study showed different result. Although population-based, this study was done at a single site, compared to 22 sites included in the LIPS cohort. Also, in the LIPS cohort we excluded the patients on ICS who were using systemic corticosteroids (SCS), after observing the higher incidence of ARDS in patients on SCS (7.7%) (2) compared to those on ICS alone (4.7%) (18). It is quite possible that some of the patients taking ICS in this single-center cohort were receiving SCS, as well. Also, in LIPS cohort studies, we used propensity score matching to account for the hidden bias and confounding, although by doing this we predisposed the results to the risk of overmatching.

Other limitations of our study need to be recognized. This was a retrospective observational study and as such it was susceptible to inherent bias and confounding. The study was done at a single academic medical center; however, all patients with ARDS in the Olmsted County, MN are treated in one of the two Mayo Clinic hospitals in Rochester, MN, which makes this population-based study. Notably, generalization of the results may be limited due to less diverse population characteristics in Olmsted County. A fraction of patients (~10%) was not included in multivariate analyses due to their lack of APACHE III score. Diagnostic ascertainment of ARDS and pneumonia also needs to be mentioned; however this particular limitation is shared with other studies on this topic. We did not have details on the use of ICS and IBA, such as compliance and duration; however this sort of information bias would be due to non-differential misclassification, which would bias results towards the null hypothesis. We used both Charlson score as well as APACHE III score to adjust for the comorbidities and severity of illness. Furthermore, most common risk factors for ARDS were accounted for. However, there could have been other confounding factors that we did not adjust for in the logistic regression analysis, therefore a potential for hidden bias remains.

Conclusion

Pre-hospital use of IBA but not ICS was independently associated with decreased incidence of ARDS and mortality once adjusted for common risk factors for ARDS, other comorbidities, and severity of illness in a population-based cohort from Olmsted County, MN. Randomized controlled trials are needed to confirm the proposed protective effects of IBA and ICS on the development of ARDS observed in preclinical and observational studies.

What is already known on this topic

Pre-clinical studies suggest separate protective roles of inhaled corticosteroids and beta agonists towards development of acute lung injury. A recent secondary analysis of a large multicenter cohort suggested possible protective role of inhaled corticosteroids in patients at risk for acute respiratory distress syndrome development by direct mechanisms, of which most common was pneumonia. Nearly 70% of patients on inhaled corticosteroids in this study were on inhaled beta agonists, as well.

What this study adds

Our population-based study demonstrates that both inhaled corticosteroids and beta agonists manifested unadjusted protective effects on development of acute respiratory distress syndrome. After adjusting for risk factors, comorbidities and severity of illness, use of inhaled beta agonists remained independently associated with the protective effect. This protective effect was more pronounced in patients with pneumonia as a predisposing factor in all unadjusted and adjusted analyses.

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Original article

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Neonatal bacterial meningitis: Results from a cross-sectional hospital based study

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Objective. The aim of the study was to determine the epidemiological characteristics of bacterial meningitis observed in neonates born in the Department of Gynaecology and Obstetrics, University Clinical Centre Tuzla, Bosnia and Herzegovina, admitted to Intensive care unit (NICU) or readmitted, because of suspected infection, after discharge from the nursery. **Subjects and methods.** This study was carried out from July 1, 2012 to June 30, 2013. During this period 4136 neonates were born. All neonates admitted to the Intensive care unit with signs and symptoms of systemic infections, and neonates readmitted to the Intensive care unit, after discharge from the nursery for sepsis work up were included in the study. **Results.** Eighteen of 200 neonates (9%) admitted or readmitted to the NICU developed meningitis. 61% cases were late onset meningitis. The overall incidence was 4.4/1000 live births. The mortality rate was 11.1%. The mean age of symptom presentation was 8.7 days. The most common clinical features were: fever, respiratory distress and jaundice. Significant risk factors for acquiring meningitis were: male gender, Caesarean delivery, stained amniotic fluid. Positive CSF finding were detected in 6/18 (33.3%) of cases. Gram-positive bacteria were more frequently responsible for confirmed meningitis. In all neonates with meningitis blood culture was examined and 5 (50%) yielded Gram-negative bacteria. **Conclusion.** The high rates of neonatal meningitis with predominant late onset may suggest nosocomial origin. Measures to improve antenatal, intrapartum and delivery care and measures during NICU hospitalisation are necessary to lower the risk of nosocomial infections.

Introduction

Acute bacterial meningitis is more frequent in the neonatal period than in any other time of life and leads to a high incidence of mortality and long term neurological sequels. The incidence of neonatal meningitis is variously calculated at between 0.25 and 0.32 per 1000 live births, depending on the inclusion criteria (1). In resource - poor countries the incidence of neonatal bacterial meningitis may be underestimated (2). In neonates with documented sepsis and in preterm infants the incidence is significantly higher. Neonates are at higher risk of meningitis because of immaturity of humoral and cellular immunity, and the absence of specific clinical signs makes diagnosis of meningitis more difficult in neonates than in older children (3). A recent review of the incidence of neonatal meningitis infections reported 0.8 to 6.1 cases every 1,000 live birth (4). Observational studies have shown that in developing countries *Klebsiella pneu-*

moniae, Serratia species and other Gram-negative bacteria are to date the leading bacterial agents of neonatal meningitis, while in Europe, America and Australia, *Group B Streptococcus* is predominant (5). There is no data on the incidence of neonatal meningitis in Bosnia and Herzegovina.

The aim of the study was to determine the incidence, aetiology, risk factors and outcome of bacterial meningitis in neonates born in Department of Gynaecology and Obstetrics, University Clinical Centre Tuzla, who were admitted to the Intensive care unit of the Department of Paediatrics for suspected infection.

Subjects and methods

This cross-sectional study involved neonates born in Department of Gynaecology and Obstetrics, University Clinical Centre, Tuzla, over a one-year period, from July 1, 2012 to June 30, 2013, who were admitted to the Intensive care unit for sepsis work up. Neonates were also included in the study after discharge from nursery and who were readmitted to the Intensive care unit with signs and symptoms of systemic infection.

Context of the study

The Department of Obstetrics and Gynecology has 4500 deliveries annually. The mothers and their babies stay in hospital for one day in normal newborn nurseries. Rooming-in was introduced in 2004, and breastfeeding is supported for well babies, but there is no feeding with fresh human milk for sick babies in the Intensive care unit.

The Neonatal Intensive Care Unit (NICU) of Department of Paediatrics provides intensive care for 18 patients. It is organized as follows: it has an average of 10 nurses, backed up by a neonatologist on a daily basis, with four intensive care physicians working in the NICU, and one on duty 24-hours. The NICU has an area of 293 m^2, it has one common and two isolation rooms, with two shared sinks and one in each isolation room. The admittance of mothers and visitors is limited to once per day for visiting and information about the baby. There is a filter before entering the ward area, equipped with a hand washing station, where all visitors are required to wash their hands. For almost all newborn, antibiotics are routinely used before entering the NICU.

Patients

In the study we enrolled 200 neonates born in the Obstetric Unit and admitted to the Intensive care unit due to various clinical problems. We also included neonates discharged from the nursery and readmitted to the Intensive care unit for sepsis work up. For each enrolled neonate with meningitis, controls were selected by the random selection method from neonates admitted to the Intensive care unit, who were without infection. Two controls were used per study case, giving a total of 36 controls.

We recorded demographic characteristics, birth weight, gestational age, gender, Apgar score, and mode of delivery. We also recorded maternal risk factors, as well as maternal hospitalisation during pregnancy, fever, and premature rupture of membranes (PROM), modality of delivery, and the presence of stained amniotic fluid.

For neonates classified as having meningitis we recorded the date of onset of the infection, symptoms at the onset of infection, microbiological data of cerebrospinal fluid (CSF), blood cultures and CSF index. Neonates were defined as having meningitis if they presented clinical symptoms of sepsis and the cerebrospinal fluid (CSF) analysis showed one or more of the following laboratory signs: 1) positive microbiological cultures 2) CSF index suggestive of bacterial process, as reported by Sarff et al. (6) con-

sidering the GA, and by Rodriguez et al. (7) considering the post-conceptional age.

Presentation during the first week of life was defined as early onset meningitis (EOM), while late onset meningitis (LOM) included presentation between the 8th and 30th postnatal days. Infection was considered nosocomial if it was diagnosed after 48 hours of maternal hospitalisation and subsequent birth, or after 48 hours of hospitalisation of the newborn (8). Blood culture was obtained for each neonate with signs of sepsis by the method described by Buttery (9). The blood specimen was further inoculated into a BACTEC Peds plus/F culture vial (BACTEC, Becton Dickinson, USA) and inoculated cultures were incubated as soon as possible in a BACTEC 9120 instrument for up to five days, as recommended by Becton Dickinson Microbiological Systems (10). PROM was defined as rupture of the membranes prior to 18 hours before delivery (11). The mortality rate was also calculated.

Ethics statement

The study was approved by the Ethics Board of University Clinical Centre Tuzla, Tuzla, Bosnia and Herzegovina. All procedures were in accordance with the Declaration of Helsinki. Informed consent was not required because all information was de-identified.

Statistical analyses

The results were analysed by arithmetic mean and standard deviation. Inferences about categorical data were analyzed using the chi-square. The association of risk factors with the development of meningitis is the frequency of each characteristic among meningitis group divided by the frequency of the same characteristic in the group of controls with no infection. This is the relative risk of infection associated with each variable. Analyses were performed by IBM SPSS Statistics 22 and MedCalc statistical software for Windows Version 13–14.10.2. P values were considered statistically significant at a value of 0.05.

Results

From July 1, 2012 to June 30, 2013 4136 neonates were born and 200 were hospitalised in the NICU. Diagnosis of meningitis was performed in 18 infants. The incidence of meningitis was 4.4/1000 live birth. The number of neonates hospitalised in the NICU with meningitis was nine, and after discharge from the nursery nine neonates were readmitted for meningitis. Risk factors for acquiring meningitis in neonates are shown in Table 1.

Birth weight (BW) and gestational age (GA) were similar between neonates with meningitis and in neonates of control group: 2867±835 g (range: 1100-4100 g) v. 2919±850 (range, 1300-4500); and 36.3 weeks (range: 30-40 weeks) v. 37.0 (range, 30-40 weeks). Half the neonates were preterm but only two showed BW ≤1500 g (11.1%). A significantly greater number of neonates with Caesarean section and with stained amniotic fluid was present in cases in comparison to the controls ($p<0.05$), as well as a greater number of male neonates ($p<0.001$).

Mean age at presentation was 8.7 days of life (range: 1-28 days). Seven of 18 the neonates with meningitis (38.9%) had EOM and 11 LOM (61.1%). Admission of neonates ≥7 days was significantly more frequent in the group of neonates with meningitis compared to those without meningitis ($p<0.001$).

The most common clinical features were fever (27.8%), respiratory distress (22.2%) and jaundice (16.7%), while other clinical findings of meningitis, such as apnoea, convulsions and irritability were less common (Table 2).

The mean leukocyte count in CSF was 498±1178 (5-5000). CSF leukocyte count was more than 1000/mm^3 in 3 (16.6%) neo-

Table 1 Risk factors for acquiring neonatal meningitis

Risk factors	Population of neonates		RR	95% CI	p
	Cases (n=18)	Controls (n=36)			
	n (%)	n (%)			
Infants					
Sex (male)	15 (83.3)	9 (25)	3.3	1.825-6.088	<0.001
BW <1501g	2 (11.1)	1(2.8)	4	0.388-41.23	>0.05
GA ≤ 32 weeks	2 (11.1)	1 (2.8)	4	0.388-41.23	>0.05
AS at 1 min ≤ 5	3 (16,7)	5 (13.9)	1.2	0.322-4.469	>0.05
AA ≥7 days	11 (61.1)	1 (2,8)	22	3.076-157.34	<0.001
Mothers					
Caesarean delivery	8 (44.4)	5 (13.9)	3.2	1.221-8.387	<0.05
Stained amniotic fluid	6 (33.3)	3 (8.3)	4	1.129-14.175	<0.05
PROM	4 (22.2)	3 (8.3)	2.6	0.667-10.663	>0.05
H ≥24 h before delivery	2 (11.1)	1 (2.8)	4	0.388-41.23	>0.05

BW=Birth weight; GA=Gestational age; AS=Apgar score; AA=Age of admission; H=Hospitalisation; PROM=Premature rupture of membrane; RR=Relative Risk; CI=95% confidence intervals.

Table 2 Clinical manifestations in newborn with meningitis

Manifestations	n (%)
Respiratory distress	4 (22.2)
Apnoea	2 (11.1)
Fever	5 (27.8)
Irritability	2 (11.1)
Convulsions	2 (11.1)
Jaundice	3 (16.7)
Total	18 (100)

Table 3 Bacteria isolated from CSF and blood in 18 neonates with meningitis

Isolates bacteria	CSF (n)	Blood (n)
Listeria monocytogenes	1	-
Acinetobacter baumannii	-	4
Enterococcus faecalis	1	2
Enterobacter cloacae	1	1
Streptococcus species	2	-
Coagulase-negative *Staphylococcus*	1	3

CSF=Cerebrospinal fluid.

nates, and more then 100/mm^3 in 8 (44.4%). Two neonates had leukocyte count within the reference range, but both had positive CSF culture and clinical manifestations of infection. The mean glucose levels, CSF glucose/blood glucose ratio and protein levels were 33±12 (5-56) mg/dl, 0.45±0.21 (0.7-0.77) and 153±65 (87-313) mg/dl. The bacterial isolates from CSF and blood in the neonates are shown in Table 3.

Bacterial CSF culture was positive in 6 neonates (33.3), showing growth of Gram-positive bacteria in 5 neonates (27.7%) and Gram-negative bacteria in 1 (5.5%). Blood culture was examined in all neonates; 10 of them had positive culture; Gram-positive bacteria were isolated from 5 (27.7%) and Gram-negative bacteria from 5 (27.7%). In two neonates, no bacteria was isolated from the CSF or blood, but pleocytosis and low glucose were found in one, and pleocytosis and high protein level in the other. The mortality rate was 11.1% in neonates with meningitis, and 4.6% in the group without infection.

Discussion

This is the first epidemiological study of neonatal meningitis in Bosnia and Herzegovina. The incidence was 4.4/1000. This

high incidence of neonatal meningitis is in keeping with the high incidence of neonatal sepsis (32.8/1000 live births, unpublished data) and with the incidence in developing countries (12, 13, 14). There is no feeding with fresh human milk for sick babies in our Intensive care unit, but it does exist in the NICU where 98% of mothers provide milk for their infants (15). This is a preventive measure to reduce the risk of infections (16). In almost all newborn, antibiotics are routinely used before entering the NICU, increasing the risk of opportunistic infection and development of antibiotic resistant organisms over time (17). Supporting breastfeeding in the NICU and antibiotic stewardship are effective clinical strategies to reduce the high incidence of neonatal sepsis and meningitis in our neonatal units.

Our data indicated the predominance of the male gender, with similar findings to Aletayeb et al. (18). The greater susceptibility of males is evident in cases of sepsis and meningitis acquired during delivery or in nurseries (19). Late onset meningitis was predominant. This finding is in keeping with the high incidence of late onset neonatal sepsis in our investigation (48.5%, unpublished data). Confirmed meningitis caused by *Listeria monocytogenes* in our study had a nosocomial origin, because in the same period a neonate was born with confirmed *Listeria monocytogenes* sepsis from a twin pregnancy and complicated Caesarean delivery. The first twin had *Listeria monocytogenes* sepsis, but the second was healthy. The neonate with *Listeria monocytogenes* meningitis had a Bruton agammaglobulinemia like predisposing risk for acquiring infection. Late onset and cross-infection of *Listeria monocytogenes* meningitis was confirmed from other authors (20, 21). Four neonates with meningitis had confirmed sepsis with *Acinetobacter baumannii*. In the same period we documented an outbreak of *Acinetobacter baumannii* in the Intensive care unit (22). The Gram-negative bacteria (*Enterobacter cloacae*), isolated from liquor and blood, *Acinetobacter baumannii* isolated from blood, and the more frequent late onset meningitis, indicate a nosocomial origin, similar to the investigation by Aletayeb et al. (18).

Caesarean delivery and stained amniotic fluid were significant risk factors for acquiring meningitis. Caesarean section is associated with more neonatal sepsis compared to normal vaginal delivery (23). In our study, stained amniotic fluid was significantly frequent in neonates with meningitis. According to the investigation by Zanella et al. (24), stained amniotic fluid was one of the obstetric risk factors for chorioamnionitis, together with frequent digital vaginal examination, and the duration of labour, with the increased risk of neonatal sepsis and meningitis. Obstetric risk factors are very important for distinguishing the origin of neonatal infection. According to the Centre for Disease Control definition, newborn infection that is the result of passage through the birth canal is considered nosocomial, and no specific time during or after hospitalisation is given to determine whether an infection is nosocomial (8). The limitation of our results according to the risk factors for acquiring meningitis might be the matching of controls with a different sex, the type of delivery or day of admission.

Fever was the most presented symptom. The late onset of meningitis and low rate of VLBW infants in our study group may be an explanation. Fever was the most common clinical feature in term-delivered neonates with meningitis in the investigation by Kavuncuoğlu et al. (13). The mortality rate was 11.1%. Compared with developed countries, it is similar (2), but the period of investigation was only one year, and there are no previous data for comparison.

In summary, in a one–year investigation, from July 1, 2012 to June 30, 2013, 4.4/1000

live newborn were diagnosed as having meningitis. The incidence of meningitis was high compared with the incidence in the developed world. The late onset of symptoms, risk factors and type of isolated bacteria may indicate a nosocomial origin. The mortality rate was 11.1%. Measures to improve antenatal, intrapartum and delivery care, and measures during NICU hospitalisation are necessary to lower the risk of nosocomial infections.

Conclusions

Important differences have been identified in the incidence, aetiology and risk factors in our study, compared with the results in developed countries. Measures to improve antenatal, intrapartum and delivery care, and measures during NICU hospitalisation are necessary to lower the risk of nosocomial infections.

What is already known on this topic

Bacterial meningitis is still a major cause of morbidity in neonates in the developing world. Differences in the epidemiology of neonatal bacterial meningitis have been identified between developed and developing countries. In our region no epidemiological study has been conducted of neonatal bacterial meningitis.

What this study adds

The results of our study do not add to general knowledge regarding the of epidemiological characteristics of neonatal bacterial meningitis, but they are important as a very careful analysis and present the epidemiology of neonatal bacterial meningitis and risk factors in this region.

Authors' contributions: Conception and design: IS, HT; Acquisition, analysis and interpretation of data: IS, MH; Drafting the article: IS, HT; Revising it critically for important intellectual content: IS, HT.

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Effect of family disintegration on age at menarche

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Key words: Age at menarche ▪ Family disintegration ▪ Psychosocial factors ▪ Familial stress ▪ Federation of Bosnia and Herzegovina.

Objective. The aim of this study was to determine the effect of psychosocial factors on the age at menarche of girls in Federation of Bosnia and Herzegovina (FBH). **Subjects and methods**. A cross-sectional study was conducted from September 2002 to May 2003 in all Cantons of the FBH. The random stratified sample included 19.803 girls aged 9.0 to 17.5 years. Data were collected using the status quo method. Probit analysis was used to estimate median age at menarche and 95% confidence intervals. **Results**. The present study shows that menarche occurred significantly earlier ($p<0.05$) in girls from dysfunctional families (median: 12.99 years, 95% confidence interval: 12.93-13.05) than in girls who grew up in intact families (median: 13.04 years, 95% confidence interval: 13.01-13.07). Analyzing separately the impact of each of family stressors on age at menarche, we found that menarcheal age was significantly lower in girls from single-mother families, whose parents are divorced, whose one parent is died and where alcoholism in family is present than in girls from intact families. Maturation was found to be earlier in girls from dysfunctional families then in those from intact families after the influence of place of residence and sibship size was eliminated. **Conclusion**. From our research we can conclude that the girls from dysfunctional families reached earlier age at menarche than their peers who grew up in normal families, and that this effect did not disappear after controlling for socioeconomic variables.

Introduction

Pubertal maturation is a complex physical process accompanied by major social and cognitive changes. This biological event is the outcome of a number of social and biological factors. The timing of puberty is influenced by genetics (1), socioeconomic status, environmental toxins, exercise, diet, weight, and the presence of chronic illness and stress (2). The relationship between stress and pubertal timing is especially intriguing. Whereas high levels of chronic and severe stress (e.g. nutritional deprivation, extreme exercise), as well as the events related to war (3, 4) are associated with delayed pubertal onset, a recent studies demonstrated that psychosocial factors such as familial/parental instability, family conflict, father absence, and lack of a traditional two-parent family structure are associated with an earlier menarche in girls (5, 6).

Draper and Harpending (7) have proposed a hypothesis that early family experiences shape the reproductive strategy which individuals will follow in later life. Whereas

father-absent girls develop behaviour profiles consistent with an expectation that paternal investment in childrearing will not be forthcoming and that pair bonds will not be enduring, those from father-present households develop as if anticipating the opposite, deferring sexual activity once they reach biological maturity while seeking to establish and maintain enduring, close, heterosexual relationships. This has been advances further by Belsky et al. (5) who have proposed a life history model of the role of psychosocial stressors in accelerating timing of puberty in girls. Also they theorized that humans have evolved to be sensitive to specific faetures of their early childhood environments, and that exposure to different environments biases children toward the development of different reproductive strategies. Children whose experiences in and around their families of origine are characterized by relatively high levels of stress (father absence, negative family relationships, lack of positive and supportive family relationships) are hypothesized to develop in a manner that speeds rates of pubertal maturation, accelerates sexual activity, and orients the individual toward relatively unstable pair bonds. Furthermore, they suggested that it was relatevely early family experiences - in the first 5-7 years of life - that shape reproductive strategy. The influence of an early family conflict and stress on acceleration of pubertal maturation has been observed by many authors, but only after the appearance of the psychosocial acceleration theory (5) its importance was emphasized. A number of studies have supported this hypothesis showing that early family disruption and separation from the father, is associated with earlier menarche (8-14).

An implication of Belsky et al. (5) model is that father-absent effects on daughters' pubertal timing should involve more than just father-absent effects; that is, quality of paternal investments should predict daughters' pubertal timing even within father-present homes. The quality of paternal care is believed to affect sexual development independent of other stressors that may be present in the family system. Parental warmth, positive family relationships, and paternal involvement in child rearing are related to a comparatively later age of menarche (15, 16). Father–daughter relationships are particularly predictive of menarche timing, and the quality of the father–daughter relationship is more strongly associated with rate of physical maturation than the quality of the mother–daughter relationship (15). More time spent by the father in child care, greater father-daughter affectionate-positivity during the early years was associated with later pubertal timing (15).

It seems that father-absent effects on pubertal timing is different from more general effects of interpersonal stress on pubertal maturation. Although mother absence is at least as stressful as father absence, it does not appear to have the same effect to daughters' pubertal timing. Mekos et al. (17) found that years of father absence but not years of mother absence had an accelerating effect on girls' pubertal maturation. Surbey (18) found that girls who grew up in father-absent homes, but not those from mother-absent homes, experienced earlier menarche than girls who grew up with both parents present.

What is the mechanism underlying earlier pubertal timing in girls in father-absent homes? It was hypothesized that exposure to unrelated adult males, especially stepfathers or mothers' dating partners would be associated with earlier pubertal maturation in girls. Research on a variety of mammalian species indicates that exposure to pheromones produced by unrelated adult male conspecifics accelerates female pubertal maturation (19). Research on humans has also provided definitive evidence of regulation of women's reproductive functioning by pheromones

(20). If human females possess physiological mechanism that accelerate pubertal maturation in response to pheromonal stimulation by unrelated adult males, then exposure to stepfathers and mother's dating partners, rather than absence of the biological father per se, should more strongly predict early pubertal timing in girls. This hypothesis is consistent with data reported by Mekos et al. (17) showing that girls in stepfather-present homes experienced faster pubertal growth than girls in single-mother homes. Consistent with this prediction, there was a significant correlation between age of daughter when an unrelated father figure first came into her life and timing of pubertal maturation. The younger the daughter at the time of the father figure's arrival, the earlier her pubertal timing (21). These results highlight a potentially important role for unrelated adult males in regulating timing of pubertal maturation in girls.

Most research on the genetic origins of menarche has been conducted using only female relatives. However, there may be paternal as well as maternal influences on age of menarche. In fact, the same genetic factors that influence fathers' likelihood to abandon marriages may contribute to an earlier age of menarche in their daughters. Shorter alleles of the X-linked androgen receptor (AR) gene are associated with aggression, impulsivity, high number of sexual partners, and divorce in males and with early age of menarche in females. These findings support a genetic explanation of the Belsky psychosocial evolutionary hypothesis regarding the association of fathers' absence and parental stress with early age of onset of menarche and early sexual activity in their daughters (22).

The aim of this study was to analyse the effect of several family stressors on the age at menarche of girls in Federation of Bosnia and Herzegovina, after controlling for socioeconomic factors which influence maturation greatly in developing countries and mask the impact of psychosocial factors.

Subjects and methods

Demographic data

Bosnia and Herzegovina (BH) is located in Southeastern Europe, covering totally 51,209.2 km^2, with a population of 3,791,622 inhabitants (23). The country is divided on two entities: Federation of Bosnia and Herzegovina (FBH) and Republica Srpska. The FBH consists of 10 federal units-cantons with a population of 2,371,603 inhabitants (23).

The BH Gross Domestic Product (GDP) total in 2003 was 7.09 billion US dollars, and the GDP per capita was 1852 US dollars, while the average net monthly salary was 524.18 BAM (288.26 US dollars using the current exchange rate) (24). In December 2003, the unemployment rate in FBH has been high, 44.01% (25).

Methods, participants and data collection

A cross-sectional study was conducted from September 2002 to May 2003 in all Cantons of the FBH. A total of 19.803 girls aged 9.0 to 17.5 years from eighty primary and thirty seven secondary schools were chosen at random from a stratified cross-sectional sample. The data used in the present study were obtained by questionnaires which provided: examination date, data and place of birth, place of residence, family size, parents' educational level, data about menarche was collected by the status quo method by a investigator who asked the girls whether or not their menarche had occurred. For family size, three categories were identified: 1, 2, and 3 children in the family. Place of residence during childhood was classified into two categories: rural and urban places. Girls were classified according to the level of

parental education into four categories: university, high school, vocational school, and elementary school. Family disintegration was assessed taking into account the following stress variables: a single-mother family, divorce or separation of the parents, death of one or both parents, parental prolonged illness, and alcoholism of one or both parents. On the basis of the data from the questionnaires, the girls were divided into two groups. The girls who lived in functional families were included in the group categorized as "Intact family", and girls who listed some of family stressors were included in the group denoted as "Dysfunctional family". Data were collected using the status quo method.

Statistical analysis

Probit analysis was used to estimate median age at menarche and 95% confidence intervals using the Probit procedure of SAS Software, version 9.00 (SAS Institute Inc., Cary, NC, USA). The statistical significance of the differences between groups was evaluated by the Student's *t*-test. A difference was considered significant when $p<0.05$.

Results

The average age at menarche of girls from families with some of childhood stressors was compared with those of girls from functional families, to establish whether girls with an experience of family stress did in fact experience menarche at an earlier age. Indeed, this study shows that menarche occurred significantly earlier ($p<0.05$) in girls from dysfunctional families (median: 12.99 years, 95% confidence interval: 12.93-13.05) than in girls belonging to intact families (median: 13.04 years, 95% confidence interval: 13.01-13.07). The median age at menarche for all FBH girls was 13.02 years (12.99-13.05, 95% CI) (26).

Analyzing separately the impact of each of family stressors on age at menarche, we found that menarcheal age was significantly lower in girls from single-mother families, whose parents are divorced, whose one parent is died and where alcoholism in family is present than in girls from intact families. In contrast, menarcheal age of girls who reported long-standing parental illness was higher, but did not differ significantly from those in intact families (Table 1).

After controlling for sibship size (two-children families), we found the similar results (Table 2). In girls from single-mother families, whose one parent is died, and whose parent is alcoholic menarche still occurred significantly earlier than in girls from intact families. Furthermore, girls whose parents are divorced also had an earlier age at menarche than girls raised in intact families, but difference was not significant. How-

Table 1 Age at menarche of girls according to the family stressors

Variable	n	Median	95% CI
Single-mother	2196	12.93*	12.84-13.02
Divorced parents	872	12.96*	12.81-13.10
Death of one parent	2026	12.98*	12.88-13.07
Death of both parents	33	-	-
Long-standing parental illness	1845	13.05†	12.95-13.15
Alcoholic	544	12.94*	12.73-13.17
Intact	14953	13.04	13.01-13.07

CI=Confidence interval; *$p<0.05$; †$p>0.05$ compared to intact family.

ever, even when the number of children in the family was held constant, menarcheal age was still higher in girls who reported long-standing parental illness compared to those from intact families.

When the size of the family was eliminated (Table 3) we found that girls growing up in dysfunctional families with one child and two children had earlier menarche than girls who were not exposed to such dysfunction. In contrast, in families with three children menarche occurred later in girls from dysfunctional families compared to those from intact families. In all three groups difference was not significant.

The group of single-mother families was the largest among the dysfunctional families and this group was selected for comparison with intact families independent of the family size which is among the most important socio-economic factors influencing on the age at menarche. Table 4 shows that girls in the groups of single-mother families with one child and two children had menarche significantly earlier compared to those from intact families. However, as in the full sample girls raised in single-mother families with three children tended to experience later menarche than girls raised in intact families, and difference was not significant.

Table 2 Age at menarche of girls from two-children families according to the family stressors

Variable	n	Median	95% CI
Single-mother	1010	12.85*	12.72-12.99
Divorced parents	628	12.87†	12.69-13.04
Death of one parent	1264	12.83*	12.71-12.95
Death of both parents	-	-	-
Long-standing parental illness	967	12.96†	12.82-13.10
Alcoholic	241	12.74*	12.41-13.02
Intact family	7487	12.95	12.90-12.99

CI=Confidence interval; *p<0.05; †p>0.05 compared to intact family.

Table 3 Age at menarche of girls from dysfunctional and intact families by family size

Number of children in the family	Dysfunctional family			Intact family		
	n	Median	95% CI	n	Median	95% CI
1	740	12.72*	12.57-12.86	864	12.81	12.66-12.95
2	2140	12.91*	12.81-13.0	7487	12.95	12.90-12.99
3	1967	13.18*	13.08-13.29	6597	13.17	13.12-13.22

CI= Confidence interval; *p>0.05 compared to intact family.

Table 4 Age at menarche of girls from single-mother and intact families by family size

Number of children in the family	Single-mother family			Intact family		
	n	Median	95% CI	n	Median	95% CI
1	455	12.66*	12.47-12.84	864	12.81	12.66-12.95
2	1010	12.85*	12.72-12.99	7487	12.95	12.90-12.99
3	729	13.24†	13.06-13.40	6597	13.17	13.12-13.22

CI=Confidence interval; *p<0.05; †p>0.05 compared to intact family.

Table 5 Age at menarche of girls from two-children dysfunctional and intact families by place of residence

Place of residance	Dysfunctional family			Intact family		
	n	Median	95% CI	n	Median	95% CI
Urban	1531	12.72*	12.62-12.83	4082	12.81	12.75-12.88
Rural	1340	13.00†	12.88-13.12	4261	13.05	12.98-13.11

CI=Confidence interval; *p<0.05; †p>0.05 compared to intact family.

Table 6 Age at menarche of girls from dysfunctional and intact families by parent's level of education

Educational level of parents	Dysfunctional family			Intact family		
	n	Median	95% CI	n	Median	95% CI
Mother						
University	252	12.54*	12.25-12.82	1447	12.92	12.81-13.03
High school	1802	12.87*	12.77-12.96	6711	12.99	12.94-13.04
Vocational school	267	13.09*	12.80-13.36	842	12.94	12.81-13.08
Elementary school	2527	13.12†	13.03-13.21	5943	13.15	13.09-13.20
Father						
University	366	12.64*	12.38-12.88	2233	12.95	12.86-13.03
High school	2137	12.91*	12.82-13.00	7152	13.02	12.97-13.06
Vocational school	1165	13.12†	12.98-13.25	3209	13.09	13.01-13.16
Elementary school	1177	13.11†	12.98-13.23	2352	13.14	13.05-13.23

Confidence interval *p<0.05; †p>0.05 compared to intact family.

Table 5 presents data on the comparison between the median age at menarche of girls from dysfunctional and intact families independent of sibship size (two-children families) and place of residence. After adjusting for these variables, we found that girls who lived in urban places and raised in two-children dysfunctional families had significantly earlier age at menarche than girls raised in two-children intact families. Menarcheal age of girls from rural places raised in dysfunctional families was lower, but did not differ significantly from those in intact families.

The results presented in Table 6 show that girls from dysfunctional families whose parents (mother or father) had a university- and high school level of education had significantly earlier age at menarche than girls from intact families. Age at menarche occurred earlier also in girls from dysfunctional families whose parents (mother or father) had elementary school degree compared to those from intact families, but difference was not significant. In contrast, girls from dysfunctional families whose parents (mother or father) had a vocational school degree had later age at menarche than girls from intact families, and difference was significant in group of girls whose mothers had this educational level.

Discussion

The aim of this study was to evaluate the impact of several family stressors on age at menarche of girls in Federation of Bosnia and Herzegovina. We hypothesized that girls from dysfunctional families, when compared to those from intact families would have earlier age at menarche. The present study indicates that girls from dysfunctional families experienced earlier age at menarche than girls who grew up in intact families.

This is consistent with other studies which have shown that father absence and early family conflict and stress are associated with earlier menarche (10, 18, 21, 27-31).

Our results have shown that age at menarche occurred significantly earlier in group of girls from single-mother families, compared to those from intact families. These data are consistent with many other studies showing that girls growing up in father-absent homes mature earlier than do other girls (9, 10, 12, 15, 21, 27-29, 32). Moreover, meta-analysis of Webster et al. (14) showed that father absence was significantly related to earlier menarche. Single parenthood may involve consequence other than father absence, specifically those of financial hardship, low social status and lack of social support. Indeed, in an analysis of four representative samples of US single-mother families, it was found that the single most important factor contributing to the difficulties experienced by children of lone parents in later life was the lower family income associated with single parenthhood (33). It is possible, however, that other pressures on these mothers such as social stigma and lack of social support may interfere with their parenting role and leave their children vulnerable to emotional problems. Weinraub (34) found that children of these mothers had more behavioural problems, poorer school performance than children from two-parent families. These negative outcomes were found to be associated with the low maternal social support and maternal stress experienced by some of the solo mothers, rather than directly related to single parenthood. In the study by Dunn et al. (35) greater maternal negativity toward the child was shown by single mothers than by mothers in two-parent heterosexual families, and found to be associated with a higher rate of behavioural problems in children. McLanahan and Sandefur (33) reported that single mothers exert less control over their children in terms of supervision and establishing rules than do mothers in two-parent families. The poorer quality of parenting shown by single mothers may be explained, in part at least, by the higher rates of psychological problems, particularly depression, found among single mothers. Depression is thought to interfere with parent's emotional availability and sensitivity to their children and also with their control and discipline of them (36).

Consistent with the previous research (26, 32), our girls whose parents are divorced had significantly earlier age at menarche than girls from intact families. These children are confronted with a series of negative events rather than a single experience. Since small procent of divorced women remarry, the economic situation of children after divorce of the parents is generally very similar to that of single-mother families. In all such cases the mother has to provide for the family and so has little time for the children who are often left at home without anybody's control. Divorce is widely viewed as a stressful life event. The most influential factor seems to be the exposure to parental conflict, which was found in a review by Amato (37) to be the most significant predictor of emotional distress in the children of divorced parents. Divorce also create adjustment difficulties for mothers, who may have raised level of depression and anxiety (38).

Girls reared in families where one or both parents are alcoholic reached menarche earlier than their peers reared in normal families, and these data are consistent with studies done in Poland (32, 39). Stressful interpersonal relationships ocurring in the family where one or both parents are alcoholic predict earlier pubertal timing in the daughter.

Menarcheal age of our girls who reported long-standing parental illness was higher than those in girls from intact families, and this was the case even when the number of children in the family was held constant. The

extent of family disruption depends on the seriousnes of the illness, the family's level of functioning before the illness, and socioeconomic considerations. In some instances, a major illness brings a family closer together; in others, even a minor illness causes significant strain. Long-term illness, even in the most stable and supportive families brings changes in family relationships. We can speculate that long-term illness in our sample decreases the socio-economic status of the family to such an extent that maturation of daughters was delayed compared to intact families, or that positive family relationships, lack of parental conflict in childrearing are related to later age of menarche.

The most obvious difficulty confronting a researcher is the presence of a number of socio-economic factors, such as urban/rural residence, family size, educational level of parents, which influence maturation greatly and mask the impact of psychosocial factors (40-42). Toromanović and Tahirović (43) have examined the effect of socioeconomic status on the age at menarche of girls in FBH and found that maturation in girls is heavily influenced by very significant inequalities in social and economical conditions in the region. This is the reason why most of the studies presented pay special attention to comparing ages at menarche of girls from dysfunctional families with those from normal families with comparable socio-economic levels. Results of our study showed that even after adjusting for these variables, girls from dysfunctional families had earlier menarche than girls from intact families.

When the size of the family was eliminated we found that girls growing up in dysfunctional families with one child and two children had earlier menarche than girls who were not exposed to such dysfunction. In contrast, in dysfunctional families with three children the low standard of living prevails in delaying maturation.

The present study showed that girls in the groups of single-mother families with one child and two children had menarche significantly earlier compared to those from intact families independent of the family size. However, the third child in a single-mother families decreases its socio-economic status to such an extent that maturation of daughters was delayed compared to intact families. These data are consistent with the study by Hulanicka (32).

In addition, maturation was found to be earlier in girls from dysfunctional families then in their peers who grew up in intact families after the influence of place of residence and sibship size was eliminated. The influence of family disintegration on age at menarche is evident independent of parental educational level with girls reared in dysfunctional families reached menarche earlier than their peers reared in normal families. We can not explain why girls from dysfunctional families whose parents (mother or father) had a vocational school degree had later age at menarche than girls from intact families, since girls whose parents have lower degree of educational level had earlier age at menarche than their peers from intact families. We may speculate that in this group the standard of living was so low causing the delay in maturation of daughters compared to those in intact families.

Limitations of the research

Although this study had a number of strengths (e.g. national sample, the evaluation of the effect of psychosocial stressors on the age at menarche after controlling for socioeconomic variables), it also has a number of limitations. The exact time or the duration in childhood when father absence occurred was not specified. Furthermore, the presence of the psychological problems, particularly depression in mothers, an exposure of girls to unrelated adult males, especially

stepfathers or mothers' dating partners, and the quality of the family relationships, especially the father–daughter relationships were not investigated.

Conclusion

The present study indicates that girls from dysfunctional families experienced earlier age at menarche than their peers who grew up in normal families supporting for the evolutionary model of pubertal timing linking stressful family environments to earlier puberty in girls. Comparing ages at menarche of girls from dysfunctional families with those of girls from normal families with comparable socio-economic levels we have eliminated the effect of socio-economic factors, which influence maturation greatly and mask the impact of psychosocial factors.

What is already known on this topic

These data are consistent with past research showing that family conflict and father absence contribute to the prediction of menarcheal age.

What this study adds

Comparing ages at menarche of daughters from dysfunctional families with those of daughters from normal families with comparable socio-economic levels we have eliminated the effect of socio-economic factors, which influence maturation greatly and mask the impact of psychosocial factors.

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Original article

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Inhaled corticosteroids and incident pneumonia in patients with asthma: Systematic review and meta-analysis

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Objectives. To systematically review all available studies on inhaled corticosteroid use and incident pneumonia in asthma patients. **Methods.** We performed a literature search from January 1, 1993, through August 15, 2015, using PubMed, Medline, CENTRAL, EMBASE, Scopus, ISI, Regulatory Documents, Web of Science and manufacturers' web clinical trial registries with multiple search terms. We included studies that compared the risk of incident pneumonia among patients utilizing and not utilizing inhaled corticosteroids. We then summarized risk estimates into two random-effect meta-analyses; one including randomized controlled trials and another one including observational studies. **Results.** Fourteen studies were estimable; ten randomized controlled trials included 19,098 participants and four observational studies included 44,016 participants. There was no heterogeneity in randomized trials and summed risk ratio demonstrated the use of inhaled corticosteroids was protective of pneumonia; risk ratio 0.74, 95% CI 0.57to 0.95, p=0.02. On the contrary, observational studies showed summed odds ratio of 1.97; 95% CI 1.87to 2.07, p<0.0001, I^2=0%, suggesting increased risk of pneumonia with use of inhaled corticosteroids in asthma patients. **Conclusions**. Inhaled corticosteroids are associated with decreased risk of incident pneumonia in patients with asthma based on meta-analysis of available randomized trials. Although observational studies in similar patients suggested higher risk of pneumonia, the inherent methodological limitations confer lower grade of confidence in these studies.

Introduction

Inhaled corticosteroids (ICS) are the most efficacious controller therapy for persistent asthma in all ages. ICS have been shown to modulate the airway inflammation underlying airway hypersensitivity to viral infections, allergens and irritants (1, 2), reduce asthma symptoms (3), and improve lung function and quality of life (3), by reducing the frequency and severity of exacerbations (4), and the risk of hospitalization (5). They may also decrease asthma mortality (6), and possibly attenuate loss of lung function in adults. The combination of an ICS and long-acting beta agonists is commonly prescribed for patients with asthma and is the preferred treatment for patients whose asthma is not controlled by an ICS alone (7, 8).

Although ICS demonstrate a favorable risk profile with minimal serious adverse effects, cataracts (9, 10), and hyperglycemia (11-13), are identified consequences complicating their use. Since the Toward a

Revolution in Chronic Obstructive Pulmonary Disease (COPD) Health (TORCH) trial (14), evidence has suggested that ICS use may be associated with an increased risk of pneumonia in patients with COPD (15-17). In contrast to COPD, several investigations failed to demonstrate an association between ICS use and the development of pneumonia in patients with asthma (18-22). Recently, McKeever et al. suggested an increased risk of pneumonia and lower respiratory tract infections (LRTI) in asthma patients utilizing ICS (23). However, this study lacked systematic and radiographic ascertainment of pneumonia, thus limiting the validity of the conclusions.

Although asthma is an independent risk factor for pneumonia (24-28), it is not clear whether ICS are further independently associated with an increased risk of pneumonia in people with asthma. Due to the conflicting results of prior investigations and their methodological limitations, we systematically reviewed the relevant medical literature and performed a meta-analysis to investigate the association of inhaled corticosteroids on the incidence of pneumonia in patients with asthma.

Methods

The review protocol was written by a senior investigator (E.F.) as a part of the Master's Program at Mayo Clinic Center for Clinical and Translational Science, CTSC 5740: Systematic Reviews and Meta-Analysis (http://www.mayo.edu/ctsa/education/current-courses-in-clinical-and-translational-science-at-mayo-graduate-school/mayo-graduate-school-course-descriptions) and was not publicly registered.

Eligibility criteria

The specific inclusion criteria for this systematic review were: (1) randomized controlled trials with minimum follow up of 4 weeks or an observational study with follow up for duration of hospitalization in participants with asthma, (2) use of any ICS medication alone or in combination with other medication as intervention versus a control group not using ICS, (3) diagnosis of incident pneumonia or lower respiratory tract infection (LRTI), or non-tuberculous mycobacterial pneumonia (NTM). Thus, reviewed studies included in our meta-analysis were RCTs and observational studies comparing the unadjusted risk of incident pneumonia (community acquired, LRTI, NTM) between patients on ICS and not on ICS. The minimal duration of exposure to ICS was not limited. Studies of patients with COPD were not eligible.

Search strategy and study selection

The search strategy was designed and conducted by a head reference librarian at Mayo Clinic, Rochester, MN. Two reviewers (V.B., M.A.M.) independently and in duplicate searched PubMed, Medline, CENTRAL, EMBASE, Scopus, Web of Science and manufacturers' web clinical trial registries (GlaxoSmithKline, AstraZeneca) using multiple search terms with no language restrictions, from January 1, 1993, through August 15, 2015. They screened all titles and abstracts identified by the preliminary library search to accrue potentially eligible studies. Then, the same reviewers independently assessed all selected full-text manuscripts for the eligibility. Disagreements regarding eligibility between 2 reviewers were resolved through consensus and after an input from a third reviewer (E.F.).

Study characteristics and quality assessment

In order to adhere to principles of sound methodological quality, we selected data col-

lection forms for RCTs based on Cochrane Collaboration risk assessment tool. For each study, we ascertained the methods for randomization sequence, allocation concealment, and identified imbalances in baseline patient characteristics, which groups were blinded, study attrition rate, and if the analyses were conducted with intention to treat (ITT). We used terms "low risk" and "high risk" of bias at the study level instead of scoring. For observational studies we adopted Newcastle-Ottawa scales for cohort and case-control studies, as applicable. Quality assessments were done independently and discrepancies were achieved by consensus. At the outcome level, we assessed risk of bias by using GRADE profiler, version 3.6 (GRADE working group).

Outcome measures

Among all studies on ICS use in asthma, those which measured and reported pneumonia (including LRTI and NTM) were analyzed in detail. Pneumonia was reported as a safety or adverse effect in all RCTs; all except one of the observational studies (23) included a more systematic assessment for pneumonia, including radiographic confirmation.

Data extraction

Two reviewers (V.B. M.A.M.) independently reviewed and abstracted data on pneumonia incidence and ICS use for each eligible RCT and observational study of patients with asthma. If there were multiple reports stemming from a single specific study database, data from the study version that provided the most robust information on pneumonia were extracted with other contributing studies included in the bibliography. When specific data was missing, corresponding authors were contacted through email, maximum of two attempts for each author. Of four authors, two replied to the first email and one of these two was able to provide required information, while two others did not respond after two attempts. Reviewers sorted data separately in all stages of study selection, data extraction, and quality assessment. All discrepancies found between 2 reviewers were resolved with consensus and after inputs from other two authors.

Quantitative data synthesis and sensitivity analysis

We analyzed data in Review Manager Software, version 5.2 (Nordic Cochrane Center, Copenhagen, Denmark), to evaluate combined risk ratio (RR) for RCTs and odds ratio (OR) for observational studies (due to inclusion of three case-control studies) with respective 95% confidence intervals (CI) using a random-effects model. All reported *p*-values are 2-sided, with significance set at less than 0.05. The statistical heterogeneity was assessed using the I^2 statistic where values of 50% or more were considered as a substantial level of heterogeneity. Where substantial statistical heterogeneity was present, we explored additionally study characteristics and to determine a potential source of heterogeneity. The subgroup analysis was defined by RCTs versus observational studies. Sensitivity analyses were planned to explore the influences on effect size by: statistical models (fixed vs random effects), individual trials and cohort versus case-control studies.

Results

Initial library search identified 463 potentially relevant citations after removing duplicates in the EndNote (version X4). We excluded 430 articles after the title and abstract reviews. Eleven additional studies were identified through the reviews of web-based pharmaceutical clinical trial registries; of these, 4 were published and 7

were unpublished. There were no disagreements between 2 reviewers at this stage. We then investigated why 7 latter studies were not included in our initial library search results and discovered that their published versions did not contain the specific term "pneumonia", which our search was based on. We subsequently performed full review of 44 studies; of those, 18 studies fulfilled the inclusion criteria for qualitative analysis and 14 of those were estimable and therefore included in 2 quantitative analyses. The flowchart is shown in Figure 1, study characteristics are shown in Tables 1 and 2 and reasons for excluded studies are shown in Table 3.

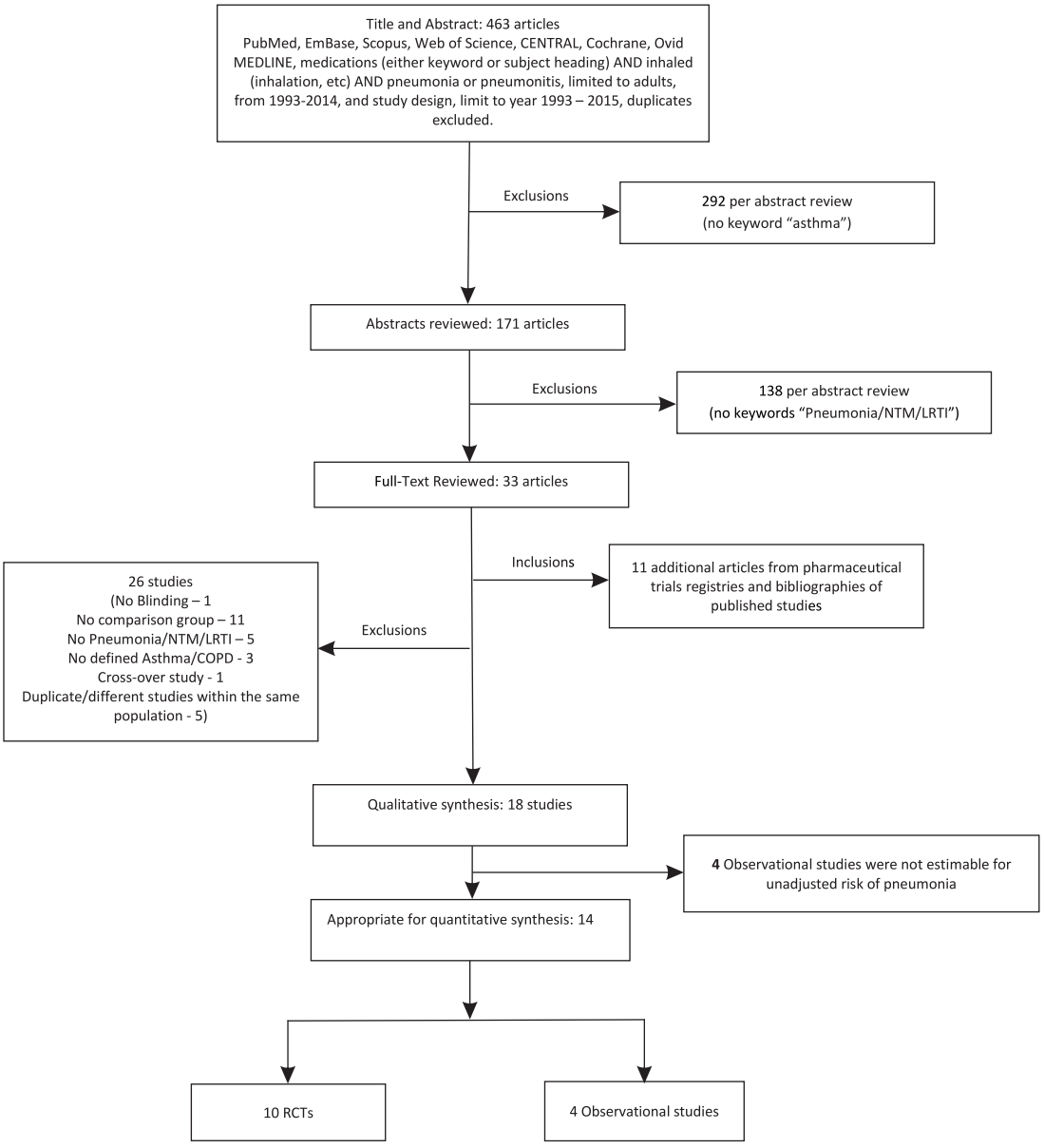

Figure 1 Study flow-chart.

Table 1a Study characteristics for RCTs*

Source	Patients	Setting	Duration†	Interventions	Enrolled/ Analyzed
ADA103575 (29)	Mild to moderate persistent asthma with age 15 years or older	Outpatient	4	Fluticasone/salmeterol 100/50 µg + Fluticasone propionate 200 µg nasal spray+ Placebo capsule	182/140
				Fluticasone/salmeterol 100/50 µg + Placebo spray + Placebo capsule	180/137
				Fluticasone/salmeterol 100/50 µg +Placebo nasal spray + Montelukast 10mg	182/129
				Placebo discus +placebo nasal spray +Montelukast 10 mg	181/138
Corren 2007 (32)	Mild to Moderate persistent asthma with age 12 years or older	Outpatient	12	Budesonide +Formoterol 160/9 µg pMDI	123/105
				Budesonide 160 µg pMDI	121/103
				Formoterol 9 µg DPI	114/79
				Placebo	122/60
FFA115285/ Busse 2014 (37)	Mild to Moderate persistent asthma with age 12 years or older	Outpatient	27	Fluticasone propionate 100 µg	115/95
				Fluticasone furoate 50 µg	117/91
				Placebo	115/77
Maspero 2013 (36)	Mild to moderate persistent asthma in adult patients (women aged 18-40 years, men aged 18-50 years)	Outpatient	52	Mometasone furoate 400 µg DPI	137/103
				Mometasone furoate 200 µg DPI	140/105
				Fluticasone propionate 250µg pMDI	147/109
				Montelukast 10 mg orally	142/111
Noonan 2006 (31)	Moderate-Severe persistent asthma with age 12 years or older	Outpatient	12	Budesonide/Formoterol 320/9 µg pMDI	124/97
				Budesonide 320µg pMDI + Formoterol 9µg DPI	115/86
				Budesonide 320µg pMDI +Placebo DPI	109/78
				Formoterol 9µg DPI + Placebo pMDI	123/60
				Placebo pMDI + Placebo DPI	125/50
Sheffer 2005 (30)	Mild persistent asthma with age 5-66 years	Outpatient	156 (3 years)	Budesonide	3630/2640
				Placebo	3591/2571
Woodcock 2011 (34)	Mild to moderate persistent with age 12 years or older	Outpatient	8	Fluticasone furoate 200 µg OD AM	105/85
				Fluticasone furoate 200 µg OD PM	103/82
				Fluticasone furoate 400 µg OD AM	111/96
				Fluticasone furoate 400 µg OD PM	113/96
				Fluticasone furoate 200 µg BID	113/96
				Placebo	101/65
Busse 2012 (35)	Mild to moderate persistent asthma that was not controlled using medium-dose ICS with age 12 years or older	Outpatient	12	Fluticasone furoate 200 µg OD Diskus/Accuhaler PM	99/81
				Fluticasone furoate 400 µg OD Diskus/Accuhaler PM	101/93
				Fluticasone furoate 600 µg OD Diskus/Accuhaler PM	107/94
				Fluticasone furoate 800 µg OD Diskus/Accuhaler PM	102/85
				Fluticasone propionate 500µg BID Diskus/ Accuhaler + Placebo OD Novel DPI	110/97
				Placebo Novel DPI	103/65
Karpel 2007 (33)	OCS dependent severe persistent asthma for at least 12 mos. with age 12 years or older	Outpatient	13 (3 months of double-blind, placebo controlled treatment phase)	Mometasone furoate MDI 400 µg BID	42/42
				Mometasone furoate MDI 800 µg BID	43/43
				Placebo (22 patient in placebo, 9 in MF-MDI 400 µg, 5 patients in MF-MDI 800 µg discontinued before 3 months due to treatment failure, 1 death in MF-MDI 400 µg before 3 month but analysis done for 123 patients as enrolled)	38/38

*Data on 26 unpublished RCTs from O'Byrne et al. (21) is not included in the table. †Duration in weeks; OCS=Ooral corticosteroids; pMDI=Delivered via metered-dose inhaler; DPI=Delivered via dry powder inhaler; AM=Morning dosing; PM=Evening dosing; OD=Once daily; BID=Twice daily.

Table 1b Quality assessment tables for RCTs*

Study ID	Risk of Bias	Grade†	Support for judgement	Funding
ADA103575 (29)	Random sequence generation (selection bias)	Low risk	Randomization criteria were assigned but not described further	GlaxoSmithKline
	Allocation concealment (selection bias)	Low risk	Allocated blindly	
	Blinding of participants and personnel (performance bias)	Low risk	Double-blind	
	Blinding of outcome assessment (detection bias) regarding pneumonia-related outcome	Low risk	Safety measures included adverse events and asthma exacerbations	
	Incomplete outcome data (attrition bias) regarding pneumonia-related outcome	High risk	Uneven withdrawal rates, no description of imputation to account for dropout	
	Selective reporting (reporting bias)	Low risk	Key expected outcome was reported	
	Systemic ascertainment of pneumonia-related outcome	High risk	Not reported	
Corren 2007 (32)	Random sequence generation (selection bias)	Low risk	By computerized randomization	AstraZeneca
	Allocation concealment (selection bias)	Low risk	Allocated done by computer-generated allocation schedule	
	Blinding of participants and personnel (performance bias)	Low risk	Double-blind (presumed participants and personnel/investigators)	
	Blinding of outcome assessment (detection bias) regarding pneumonia-related outcome	Low risk	All ranges/outcomes were pre specified before study unblinding as part of the statistical analysis plan	
	Incomplete outcome data (attrition bias) regarding pneumonia-related outcome	High risk	Uneven withdrawal rates, no description of imputation to account for dropout	
	Selective reporting (reporting bias)	Low risk	Key expected outcome was reported	
	Systemic ascertainment of pneumonia-related outcome	High risk	Not reported	
FFA115285/ Busse 2014 (37)	Random sequence generation (selection bias)	Low risk	Randomized in accordance with a central randomization schedule	GlaxoSmithKline
	Allocation concealment (selection bias)	Unclear risk	Not reported	
	Blinding of participants and personnel (performance bias)	Low risk	Double-blind	
	Blinding of outcome assessment (detection bias) regarding pneumonia-related outcome	Low risk	Safety endpoints were incidence of adverse events (AEs) and of protocol-defined severe asthma exacerbations during the treatment period	
	Incomplete outcome data (attrition bias) regarding pneumonia-related outcome	High risk	The withdrawal rates were very high compared to the number of events for the different outcomes	
	Selective reporting (reporting bias)	Low risk	Key expected outcome was reported	
	Systemic ascertainment of pneumonia-related outcome	Low risk	Suspected pneumonia was confirmed by X-ray	
Maspero 2013 (36)	Random sequence generation (selection bias)	Low risk	Randomization was centrally administered by using an interactive voice response system	Merck & Co Inc.
	Allocation concealment (selection bias)	Unclear risk	Not reported	
	Blinding of participants and personnel (performance bias)	Low risk	Double-blind	
	Blinding of outcome assessment (detection bias) regarding pneumonia-related outcome	Low risk	Rescue medication use and symptom scores were documented, and the patients were examined at all visits	

Continuation of Table 1b Quality assessment tables for RCTs

Study ID	Risk of Bias	Grade†	Support for judgement	Funding
Maspero 2013 (36)	Incomplete outcome data (attrition bias) regarding pneumonia-related outcome	High risk	The withdrawal rates were very high compared to the number of events for the different outcomes	Merck & Co Inc.
	Selective reporting (reporting bias)	Low risk	Key expected outcome was reported	
	Systemic ascertainment of pneumonia-related outcome	High risk	Not reported	
Noonan 2006 (31)	Random sequence generation (selection bias)	Low risk	Randomization was performed using a computer generated allocation schedule and stratified by asthma severity, based on the daily dose of ICS before entering the study	AstraZeneca
	Allocation concealment (selection bias)	Low risk	Computer generated allocation	
	Blinding of participants and personnel (performance bias)	Low risk	Double-blind	
	Blinding of outcome assessment (detection bias) regarding pneumonia-related outcome	Low risk	Safety was evaluated based on adverse events, laboratory evaluations, vital signs, ECGs, 24-hour Holter monitoring and physical examinations	
	Incomplete outcome data (attrition bias) regarding pneumonia-related outcome	High risk	The withdrawal rates were very high compared to the number of events for the different outcomes	
	Selective reporting (reporting bias)	Low risk	Key expected outcome was reported	
	Systemic ascertainment of pneumonia-related outcome	High risk	Not reported	
Sheffer 2005 (30)	Random sequence generation (selection bias)	Low risk	Randomization was stratified into two strata according to age; age less than 11 years or age at least 11 years Within each stratum, patients were randomized in blocks of ten, five in each treatment group	AstraZeneca
	Allocation concealment (selection bias)	Low risk	Randomly allocated	
	Blinding of participants and personnel (performance bias)	Low risk	Double-blind	
	Blinding of outcome assessment (detection bias) regarding pneumonia-related outcome	Unclear risk	Safety outcomes of the START clinical study included all AEs and asthma-related events from spontaneous reporting and patient's responses to standard questioning during the 3-year study period (6 and 12 weeks after randomization and then every 3 months up to 3 years)	
	Incomplete outcome data (attrition bias) regarding pneumonia-related outcome	High risk	The withdrawal rates were very high compared to the number of events for the different outcomes	
	Selective reporting (reporting bias)	Low risk	Key expected outcome was reported	
	Systemic ascertainment of pneumonia-related outcome	High risk	Not reported	
Woodcock 2011 (34)	Random sequence generation (selection bias)	Low risk	The central randomization schedule was generated by the sponsor using a validated computerized system	GlaxoSmithKline
	Allocation concealment (selection bias)	Low risk	Allocated randomly by using Registration and Medication Ordering System (RAMOS), an automated, interactive telephone based system	
	Blinding of participants and personnel (performance bias)	Low risk	Double-blind	

Continuation of Table 1b Quality assessment tables for RCTs

Study ID	Risk of Bias	Grade†	Support for judgement	Funding
Woodcock 2011 (34)	Blinding of outcome assessment (detection bias) regarding pneumonia-related outcome	Low risk	The following safety endpoints were evaluated: incidence of adverse events (AEs) and serious AEs (SAEs), vital signs, hematology, clinical chemistry, and urinalysis parameters, oropharyngeal examinations, and withdrawals due to worsening asthma. AEs/SAEs were coded using the Medical Dictionary for Regulatory Activities	GlaxoSmithKline
	Incomplete outcome data (attrition bias) regarding pneumonia-related outcome	High risk	The withdrawal rates were very high compared to the number of events for the different outcomes	
	Selective reporting (reporting bias)	Unclear risk	Authors used upper respiratory tract infection and respiratory tract infection separately in AE. We presumed RTI was LRTI	
	Systemic ascertainment of pneumonia-related outcome	High risk	Not reported	
Busse 2012 (35)	Random sequence generation (selection bias)	Low risk	The central randomization schedule was generated by the sponsor using a validated computerized system	GlaxoSmithKline
	Allocation concealment (selection bias)	Low risk	Allocated randomly by using Registration and Medication Ordering System (RAMOS), an automated, interactive telephone based system	
	Blinding of participants and personnel (performance bias)	Low risk	Double-blind	
	Blinding of outcome assessment (detection bias) regarding pneumonia-related outcome	Low risk	Adverse events (defined using the Medical Dictionary for Regulatory Activities V.11) were documented during the 8-week treatment period	
	Incomplete outcome data (attrition bias) regarding pneumonia-related outcome	High risk	The withdrawal rates were very high compared to the number of events for the different outcomes	
	Selective reporting (reporting bias)	Low risk	Key expected outcome was reported	
	Systemic ascertainment of pneumonia-related outcome	High risk	Not reported	
Karpel 2007 (33)	Random sequence generation (selection bias)	Low risk	Randomization criteria were assigned but not described further	No source of funding/support mentioned in article
	Allocation concealment (selection bias)	Unclear risk	Not reported	
	Blinding of participants and personnel (performance bias)	Low risk	Double-blind	
	Blinding of outcome assessment (detection bias) regarding pneumonia-related outcome	Low risk	All patients were monitored for adverse events and changes in physical findings, vital signs, hematological and blood chemistry profiles, and electrocardiographic profiles	
	Incomplete outcome data (attrition bias) regarding pneumonia-related outcome	High risk	The withdrawal rates were very high compared to the number of events for the different outcomes	
	Selective reporting (reporting bias)	Low risk	Key expected outcome was reported	
	Systemic ascertainment of pneumonia-related outcome	High risk	Not reported	

*Data on 26 unpublished RCTs from O'Byrne et al. (21) is not included in the table; †Graded by authors; LRTI=Lower respiratory tract infection; NTM= Nontuberculous pulmonary mycobacteriosis / non-tuberculous mycobacterial pneumonia.

Table 2a Study characteristics for observational studies

Source	Type of study	Patients	Setting	Duration	Interventions	Subjects (n)	Risk of bias	
Almirall 2010 (20)	Case control study	Diagnosis of community-acquired pneumonia patient with three chronic respiratory diseases that require inhaled therapy were included: chronic bronchitis, COPD and asthma with age 14 years or older	Outpatient	1 year (1999-2000)	Asthma ICS	30	Selection	Low
					Asthma Non-ICS	344	Indication	High
Andrejak 2013 (41)	Population based case-control study	Adult patient (age 15 years or older) with microbiologically confirmed NTM pulmonary disease with any chronic respiratory diseases	Outpatient	12 years (1997-2008)	Asthma ICS	30	Selection	Low
					Asthma Non-ICS	3	Indication	High
Festic 2014 (22)	Cohort study	Adult patients hospitalized with at least 1 major risk factor for acute respiratory distress syndrome	Inpatient	Hospitalization Mar. 2009-Aug. 2009	Asthma ICS	149	Selection	Low
					Asthma Non-ICS	291	Indication	High
Mckeever 2013 (23)	Nested Case control study	Adult asthma patients (age 18 to 80) with pneumonia or lower respiratory tract infection	Outpatient	3 years (2004-2007)	Asthma ICS	15594	Selection	High
					Asthma Non-ICS	27575	Indication	High
To M 2004 (19)	Retrospective cohort study	Asthma patients who required hospitalization for community-acquired pneumonia with age 16 year or older	Inpatient	Hospitalization 13 years (1989-2001)	Asthma ICS	37	Selection	Low
					Asthma Non-ICS	25	Indication	High
Ferrer 2014 (42)	Prospective observational cohort study	Patients aged ≥16 years hospitalized with a diagnosis of CAP	Inpatient	Hospitalization Jan. 2003-Oct. 2005	Asthma ICS	12	Selection	Low
					Asthma Non-ICS	28	Indication	High
Sellares 2013 (40)	Prospective observational cohort study	Patients admitted to the emergency room with a diagnosis of CAP with age 16 year or older	Inpatient	Hospitalization Jan. 1997-Jul. 2008	Asthma ICS	81	Selection	Low
					Asthma Non-ICS	72	Indication	High
Terraneo 2014 (43)	Prospective observational cohort study	Adult patients hospitalized with CAP	Inpatient	Hospitalization in Jan. 2000-Dec. 2011	Asthma ICS	72	Selection	Low
					Asthma Non-ICS	67	Indication	High

COPD=Chronic obstructive pulmonary disease; ICS=Inhaled corticosteroids; CAP=Community-acquired pneumonia.

Table 2b Quality assessment tables for observational studies

Case Control studies

Study	Selection	Comparability	Exposure
Almirall 2010	3/5	1/2	4/4
Andrejak 2013	3/5	1/2	3/4
Mckeever 2013	3/5	1/2	3/4

Note: Points assessed in lieu of actual over possible stars per Quality Assessment Scale used (Supplementary material).

Cohort studies

Study	Selection	Comparability	Outcome
Ferrer 2014	3/5	2/2	3/3
Festic 2014	5/5	2/2	3/3
Sellares 2013	3/5	2/2	3/3
Terraneo 2014	3/5	2/2	3/3
To m 2004	3/5	2/2	3/3

Note: Points assessed in lieu of actual over possible stars per Quality Assessment Scale used (Supplementary material).

Table 3 Excluded studies

Study ID	Reason for exclusion
D589IL00001/NCT01232348	No blinding, no control group
Beasley 2015	No control group
D5890L00008/NCT00242411	No control group
D5890L00009/NCT00290264	No control group
Hojo 2012	No control group
Lin 2015	No control group
Lukaszyk 2011	No control group
Lukaszyk 2011-2	No control group
SAM 106538/NCT00363480	No control group
Peters SP 2010	No control group
Teichert 2014	No control group
Woodcock 2014	No control group
Corren 2013	No pneumonia reported
HZA106827/ NCT01165138/ Bleecker 2014	No pneumonia reported
Nathan 2012	No pneumonia reported
Pearlman 2013	No pneumonia reported
Price 2013	No pneumonia reported
Cheng 2013	Cross-over design
Almirall 2008	Duplicate publication, same study population as in Almirall 2010
Almirall 2013	Duplicate publication, same study population as in Almirall 2010
D5254C00111/NCT00641914/O'Byrne 2009	Duplicate publication, same study population as in Sheffer 2005
NCT01232335	Duplicate publication, same study population as in D589IL00001/NCT01232348
Pauwels 2003	Duplicate publication, same study population as in Sheffer 2005
Almirall 1999	No distinction between Asthma versus COPD cases
Eurich 2013	No distinction between Asthma versus COPD cases
Farr 2000	No distinction between Asthma versus COPD cases

Randomized controlled trials

There were 9 RCTs (29-37) and one additional study (21) that reported results of 26 unpublished pharmaceutical trials on different formulations of budesonide compared to placebo. Together, these studies included 19,098 patients, of whom 12,008 received ICS and 7,090 did not. The duration of trials ranged from 4 weeks to 3 years, with median duration of 12 weeks. All published RCTs were deemed high quality studies based on the sequence generation, allocation concealment and double-blinding (Table 1b). At the outcome-level, RCTs were judged to be at high risk of bias because ascertainment of pneumonia was not performed systematically (Table 4). However, this bias would be non-differential as in blinded RCTs it would then similarly affect both intervention and control groups.

Table 4 Outcome-level quality assessment and summary of findings (GRADE)

Pneumonia with ICS versus non-ICS											
Quality assessment							Summary of findings				
Participants (studies)	Risk of bias	Inconsistency	Indirectness	Imprecision	Publication bias	Overall quality of evidence	Study event rates (%)		Relative effect (95% CI)	Anticipated absolute effects	
							Non-ICS	ICS		Non-ICS	Risk with ICS (95% CI)
RCT											
19,098 (10 studies)	High[1]	No serious inconsistency	No serious indirectness	No serious imprecision[2]	Undetected	⨁⨁⨁⊖ Moderate[1,2] due to risk of bias	128/ 7,090 (1.8%)	116/ 12,008 (1%)	RR 0.74 (0.57 to 0.95)	Study population	
										18 per 1000	5 fewer per 1000 (from 1 fewer to 8 fewer)
										Moderate	
										3 per 1000	1 fewer per 1000 (from 0 fewer to 1 fewer)
Observational											
44,016 (4 studies)	Very high[3,4,5]	No serious inconsistency	No serious indirectness	No serious imprecision	Undetected	⨁⊖⊖⊖ Very low[3,4,5] due to risk of bias	3,733/ 28,213 (13.3%)	3,517/ 15,803 (22.3%)	OR 1.97 (1.87 to 2.07)	Study population	
										133 per 1000	99 more per 1000 (from 90 more to 108 more)
										Moderate	
										333 per 1000	163 more per 1000 (from 150 more to 175 more)

[1]Limited pneumonia ascertainment; [2]Although several trials had wide confidence intervals, these represented less than 5% of the weight; [3]Case control and historical cohort designs; [4]Unaccounted step up in ICS therapy due to persistent respiratory symptoms preceding the diagnosis of pneumonia; [5]One study carried 98% of overall weight.

The estimated overall unadjusted risk of pneumonia with the use of ICS in RCTs, was in protective range; RR 0.74, 95% CI 0.57 to 0.95, p=0.02, without any heterogeneity (Figure 2). As the details on 26 unpublished RCTs reported in the single study by O'Byrne et al. (21) were not available, we performed a sensitivity analysis. When we excluded results of O'Byrne study (21), the confidence interval extended to 1 (95% CI

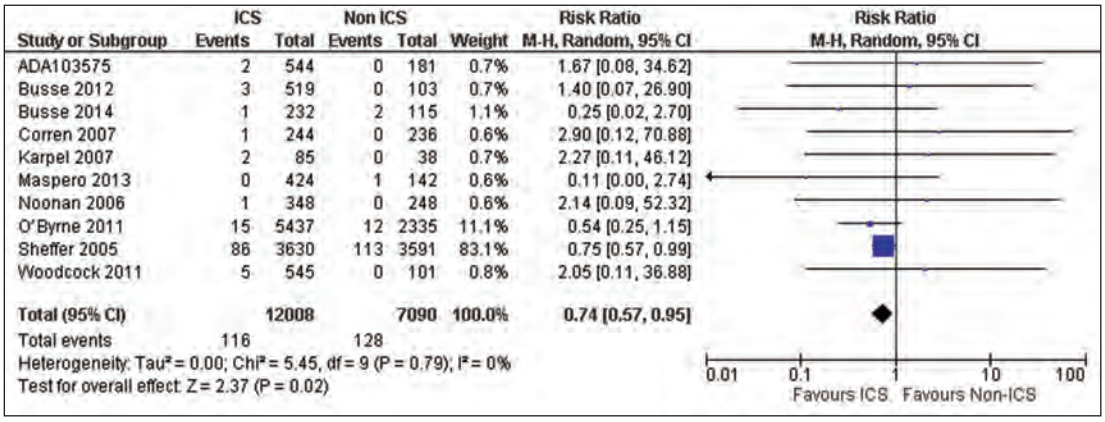

Figure 2 Meta-analysis of RCTs for incident pneumonia.

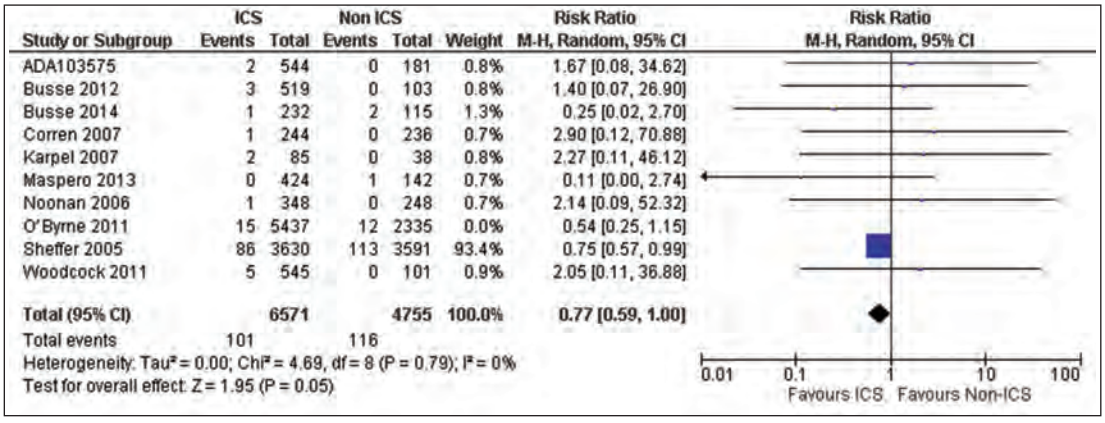

Figure 3 Sensitivity analyses of RCT data - A) Without O'Byrne 2011 study data.

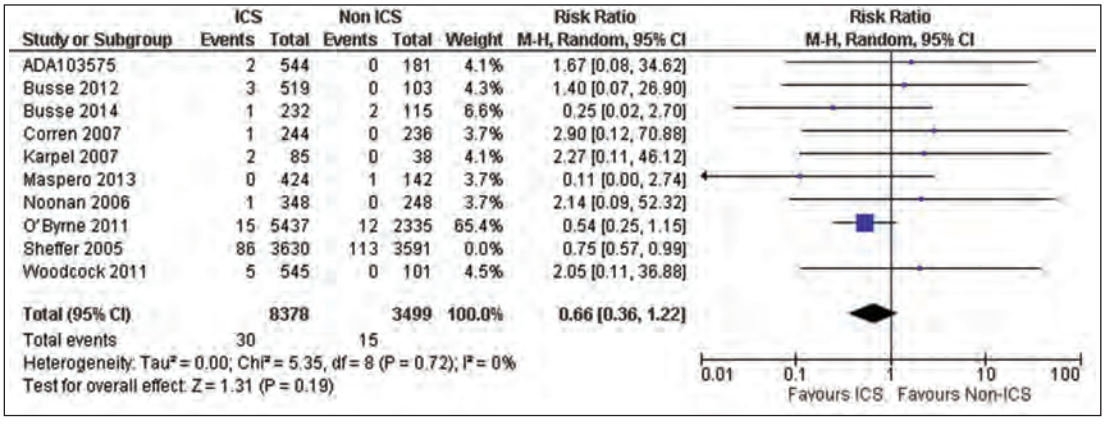

Figure 3 Sensitivity analyses of RCT data - B) Without Sheffer 2005 study data.

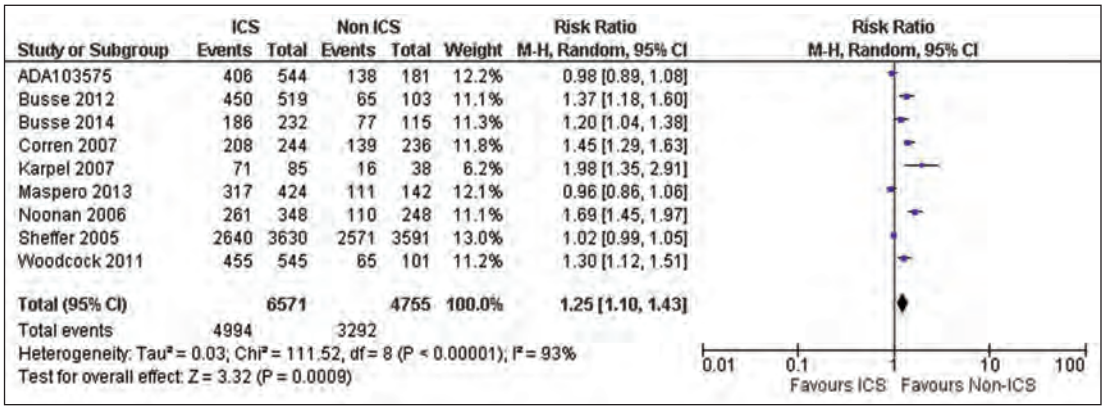

Figure 4 Study completion rates comparing ICS and non-ICS groups.

0.59-to 1, p=0.05) (Figure 3A). As a result, the weight of study by Sheffer et al. (30) in the meta-analysis consequently increased from 83.1% to 93.4%. This study was one of the three published START trial reports (30, 38, 39). Once the study by Sheffer et al. was removed because of the overly dominant weight, in a subsequent sensitivity analysis the pre-hospital use of ICS in asthma patients did not show significant protective effect for pneumonia any longer (RR 0.66, 95% CI 0.36 to 1.22, p=0.19) possibly due to loss of power, as 80% of pneumonia cases were consequently excluded from the analysis (Figure 3B).

We also assessed the study completion rates between the ICS and non-ICS groups in the RCTs. Eight RCTs were estimable and the trial completion rate was higher in the non-ICS than in the ICS group; RR 1.25; 95% CI 1.10to 1.43, p=<0.001; I^2=93% (Figure 4). Only 2 RCTs reported occurrence of deaths; there were total of 13 deaths, 5 in ICS and 8 in non-ICS group, respectively (30, 33).

Observational studies

We initially included 8 observational studies (19, 20, 22, 23, 40-43). Five cohort studies (19, 22, 40, 42, 43) excluded patients on systemic corticosteroids and three case-control studies (20, 23, 41) adjusted for systemic corticosteroid use. Two studies assessed risk of outpatient pneumonia (20, 23), five assessed pneumonias requiring admission to the hospital (19, 22, 40, 42, 43), and one study (41) assessed risk of non-tuberculous mycobacteriosis by using NTM index rate. Although ascertainment of pneumonia in observational studies was more systematic by using not only clinical diagnosis but radiographic assessment as well, all observational studies were judged to be at very high risk of bias (Tables 2 and 4). Four observational studies were not estimable for unadjusted risk of pneumonia as they included only patients with pneumonia so the unadjusted differential risk of ICS could not be estimated. The remaining 4 estimable studies included 44,016 patients, of whom 15,803 were on ICS and 28,213 were not on ICS. The risk of incident pneumonia was found to be increased; OR 1.97; 95% CI 1.87to 2.07, p<0.0001, with no observed heterogeneity (Figure 5). Three studies were case-control studies and one was secondary analysis of a large cohort (Table 2). Of note, recently published study by McKeever et al. (23) carried almost complete weight (98%) in this meta-analysis. Once this study was excluded in a sensitivity analysis (Figure 6), the estimated effect decreased appreciably to OR 1.57; 95% CI 1.09 to 2.25). Exclusion of a single study that assessed risk of NTM (41) did not change the results significantly.

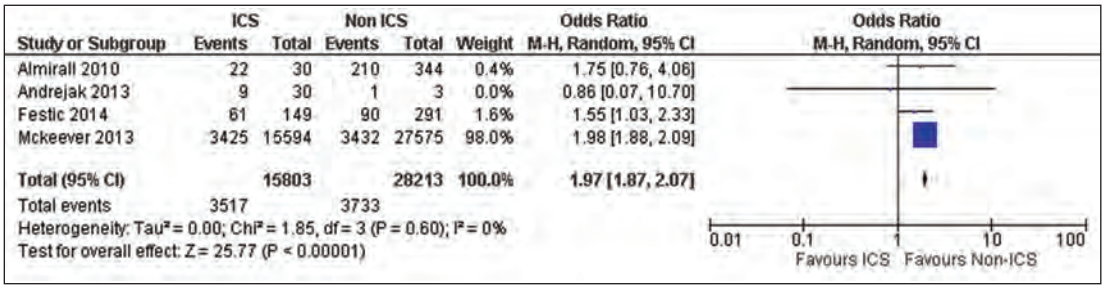

Figure 5 Meta-analysis of observational studies for incident pneumonia.

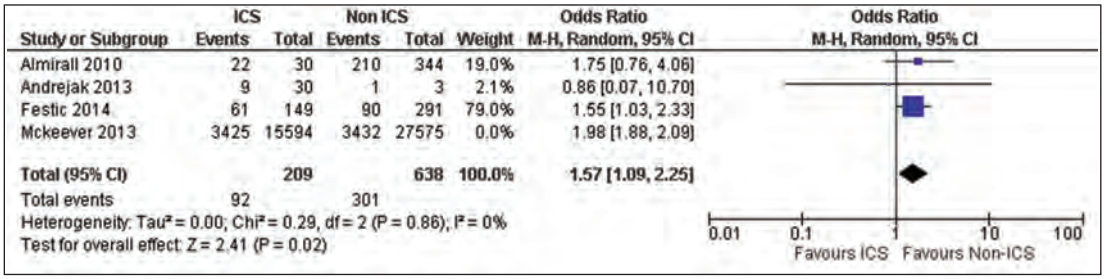

Figure 6 Sensitivity analysis for observational studies.

Mortality was reported only in two observational studies (20, 43); 2 in ICS and 6 in non-ICS group. The observed low mortality rates in both RCTs and observational studies precluded performance of pooled analysis.

Discussion

Based on available RCTs, this meta-analysis suggests that ICS are associated with decreased risk of incident pneumonia in asthma patients for the duration of respective clinical trials. Although observational studies suggested increased risk of incident pneumonia in similar patients using ICS, the inherent methodological limitations and higher risk of bias conferred lower grade of confidence in the findings of the observational studies. To our knowledge, this is the first systematic review and meta-analysis of all available RCTs and observational studies assessing the association between ICS use and risk of incident pneumonia in asthma patients.

There has been a clinical controversy regarding the risk of pneumonia in patients on ICS with COPD and asthma. Since the TORCH study reported increased incidence of pneumonia among COPD patients in 2007 (14), several well-designed trials and meta-analyses demonstrated the similar risk (44-49). However, the risk for developing community acquired pneumonia among asthma patients on ICS did not appear to be substantially increased (18-22).

Our meta-analysis of RCTs suggests that ICS are associated with decreased risk of incident pneumonia in asthmatic patients. There was no heterogeneity and there was overall less risk of bias compared to observational studies. It is uncertain why the use of ICS may be associated with an increased risk of incident pneumonia in patients with COPD but not asthma. It has been hypothesized that ICS more efficiently reduce airway inflammation, segmental atelectasis, mucoid impaction, and thus, subsequent pneumonia in patients with asthma compared with those with COPD (50). Additionally, patients with COPD are commonly of older age and have a greater burden of comorbid diseases than asthmatics, which are recognized risk fac-

tors for pneumonia. Thus, higher observed pneumonia rates in COPD patients compared to patients with asthma may be partly explained by difference in age and comorbidities. Importantly, two RCTs (29, 34) in asthma patients were shorter than 12 weeks, while the shortest clinical trials in COPD patients were 24-week long. It is conceivable that any proposed medication adverse effect could become more apparent in the studies of longer duration. Moreover, we observed higher study completion rate among non-ICS patients compared to ICS patients in eight estimable RCTs in asthma patients, however with very high heterogeneity.

We believe that it is safer to conclude that the incident risk of pneumonia with the ICS use in RCTs of asthma patients was not increased, rather than it was decreased. Although the primary meta-analysis of RCTs suggested that ICS might carry protective effect, once we excluded the trial by Sheffer et al. (30) in a sensitivity analysis, the protective effect was not statistically significant any longer. A possible explanation for the "overinflated" protective effect observed in this particular study could have been incorrect allocation of respiratory events known to be improved by ICS as pneumonia adverse events. These events could have been: segmental atelectasis due to mucous impaction that is more often seen in children with poorly controlled asthma; increased cough and mucous production; or mild asthma exacerbations. This interpretation is supported by the fact that the most frequent reporting of pneumonia adverse events was in children aged 5 to 11 years, in whom atelectasis is more frequently seen as a consequence of asthma exacerbation than in adult patients (21). Since ICS effectively improve flow limitation in asthma patients, the patients on ICS could have had less incorrectly allocated respiratory events as pneumonia. Moreover, study by Sheffer et al. included only preparations of budesonide, which has been shown previously to have more rapid clearance from the airways and to be less potent than fluticasone (51, 52). However, this is only speculative and would require future well designed prospective studies with a strict definition for pneumonia to fully resolve.

On the contrary, the pooling of observational studies suggested higher risk of pneumonia in patients with asthma. A single study by McKeever et al. carried almost complete weight in this meta-analysis (23). This recently published case-control study showed that asthma patients admitted with pneumonia were more likely to have prescription for ICS than the control subjects in the preceding 90 days; they were also more likely to use reliever inhalers and oral steroids in the previous year. The investigators used clinical diagnosis of pneumonia and did not necessarily base it on the findings on chest radiographs. Therefore, we propose that a substantial number of patients who were retrospectively included in this study (and other similar observational studies) may have had unrecognized pneumonia leading to persistent respiratory symptoms prompting increased asthma therapy containing ICS. The authors recognize this limitation of their study (23) and concluded that the prescribers should consider possibility of incipient infection rather than underlying asthma being responsible for the worsening respiratory symptoms before prescribing or increasing ICS dose. The similar was also previously demonstrated in a study on COPD patients by Calverley et al. (47). The data interpretation from this study's daily record cards suggested identical numbers of de novo pneumonias in both ICS and non-ICS arms, but more unresolved exacerbations preceding pneumonia events in the ICS-treated COPD patients. Finally, it is possible that some patients with concomitant COPD were included in the observational studies of asthma patients, which was likely not the case in RCTs.

Although the overall pneumonia ascertainment was more systematic in most of the observational studies compared to RCTs, the resulting overall grade of confidence was lower for observational studies compared to the randomized trials due to very high risk of bias (mainly indication and selection bias) (Table 4). Of note, using GRADE profiler for outcome-level quality assessment may be associated with the rater-dependent subjectivity. After cautiously analyzing and weighing all available pertinent factors, we proposed "moderate" and "very low" quality grades for analyzed RCTs and observational studies, respectively. The limitation of RCTs lacking systematic ascertainment of pneumonia would be an example of non-differential bias; therefore we labeled this as "high" rather than "very high" risk of bias. However, even if RCTs were downgraded to "low" quality given concerns with pneumonia ascertainment, the overall resulting grades of confidence would still favor RCTs rather than observational studies on the topic, which were not necessarily population-based observational studies.

Our meta-analysis has several limitations, some of which are attributable to methodological shortcomings in the studies included. We were somewhat surprised with the relatively small number of studies retrieved by our search. The reason for this could be that either study investigators did not systematically measure incident pneumonia events (including radiographic assessment), or less likely they did not report those in their publications. Therefore, our review is prone to the reporting bias as we depended solely on the reporting of outcomes. There is also publication bias risk as all RCT were pharmaceutical industry-funded. However, we reviewed the clinical trials registry and included both published and unpublished studies. The resulting funnel plot of RCTs did not suggest publication bias (Supplemental material). Although the risk of pneumonia was unadjusted, the large number of patients included in the meta-analysis partially alleviated this concern. We did not have individual-patient data, so we could not detect any differences in pneumonia based on demographics, asthma severity or presence of comorbidities. The time of follow-up in included studies differed widely, which may have also impacted results. We considered all patients on ICS as ICS users regardless of ICS being used alone or in combination with another medication. Also, non-ICS users were considered all patients not on ICS regardless of use of additional medications, such as long-acting beta agonists or placebo. This is justified by our stated main intention of assessment for the overall association of ICS and pneumonia. Only future prospective trials of ICS designed to systematically assess and monitor pneumonia as a pre-specified outcome using an objective pneumonia definition could alleviate the above-mentioned limitations.

Conclusion

Results from our meta-analysis on available RCTs suggest that ICS use in patients with asthma was associated with decreased risk of pneumonia. On the contrary, a meta-analysis of observational studies suggested a higher risk of pneumonia in similar patients; however, the grade of confidence in this subgroup's results is lower due to inherent methodological limitations. The design of future prospective trials of ICS should include systematic assessment and monitoring of pneumonia as a pre-specified outcome.

What is already known on this subject

Inhaled corticosteroids are the mainstay of asthma treatment for all ages. Their use has been previously associated with increased risk of pneumonia among COPD patients. It is uncertain if there is an association between long-term use of inhaled corticosteroids and the incident pneumonia among asthmatic patients.

What this study adds

This is the first systematic review on use of inhaled corticosteroids and incident pneumonia in asthmatic patients. It includes randomized clinical trials as well as observational studies, which were pooled in the two separate meta-analyses. While randomized clinical trials showed decreased risk of incident pneumonia, this risk was increased in observational studies, which were at higher risk of bias and conferred lower grade of confidence.

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Supplementary material

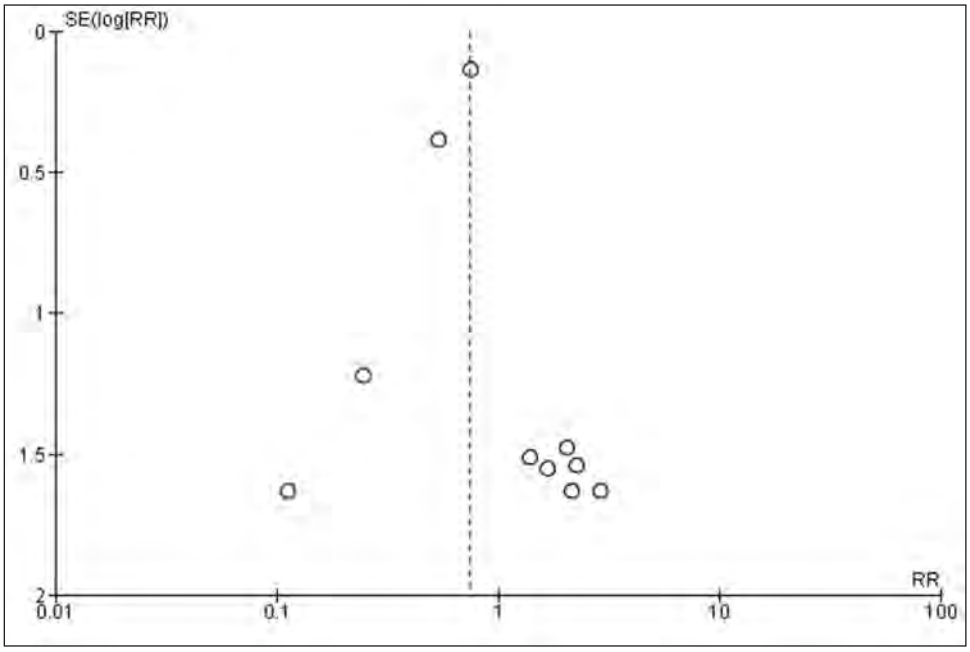

Figure S1 Funnel plot of RCTs suggesting no significant publication bias.

Search strategy (complete copied electronic search sequence)

((corticosteroid* OR beclomethasone OR triamcinolone* OR flunisolide OR budesonide OR fluticasone OR mometasone OR ciclesonide) AND (inhal* OR bronchodilat*) AND pneumoni* AND (los OR hospitali* OR ventilat* OR "length of stay")) NOT MEDLINE[sb] PubMed 15 August 2015 = 17

Ovid MEDLINE (R) In-Process & Other Non-Indexed Citations and Ovid MEDLINE (R) 1946 to Present			
	Searches	Results	Search Type
1	(beclomethasone or triamcinolone* or flunisolide or budesonide or fluticasone or mometasone or ciclesonide).mp.	21486	Advanced
2	exp glucocorticoids/ or 1	174737	Advanced
3	exp pneumonia/ or pneumoni*.mp.	177636	Advanced
4	2 and 3	3240	Advanced
5	(inhal* or ics).mp. or administration, inhalation/	139726	Advanced
6	4 and 5	308	Advanced
7	limit 6 to ("young adult (19 to 24 years)" or "adult (19 to 44 years)" or "young adult and adult (19-24 and 19-44)" or "middle age (45 to 64 years)" or "middle aged (45 plus years)" or "all aged (65 and over)" or "aged (80 and over)")	134	Advanced
8	6 and adult*.mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier]	77	Advanced
9	7 or 8	145	Advanced
10	limit 9 to (clinical trial, all or clinical trial, phase i or clinical trial, phase ii or clinical trial, phase iii or clinical trial, phase iv or clinical trial or comparative study or controlled clinical trial or evaluation studies or meta analysis or multicenter study or observational study or randomized controlled trial)	56	Advanced
11	9 and ("case adj control*" or observational* or cohort* or retrospective* or prospective*).mp.	30	Advanced
12	10 or 11	72	Advanced
13	limit 12 to yr="1993 - 2015"	65	

Manually excluded studies on COPD patients; Central=48, same strategy 1993-2015; Embase 1988 to 2015.

#	Searches	Results	Search Type
1	(beclomethasone or triamcinolone* or flunisolide or budesonide or fluticasone or mometasone or ciclesonide).mp.	46276	Advanced
2	exp pneumonia/ or pneumoni*.mp.	262692	Advanced
3	exp asthma/ or asthma*.mp. or copd.mp. or "chronic obstructive".mp. or pulmonary disease, chronic obstructive/	264244	Advanced
4	exp glucocorticoid/ih	9549	Advanced
5	(1 and inhal*.mp.) or 4 [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword]	16826	Advanced
6	2 and 3 and 5	830	Advanced
7	limit 6 to (adult <18 to 64 years> or aged <65+ years>)	235	Advanced
8	exp case control study/ or exp case study/ or exp clinical trial/ or exp "clinical trial (topic)"/ or exp major clinical study/ or exp prospective study/ or exp retrospective study/	3160131	Advanced
9	7 and 8	138	Advanced
10	7 and (cohort* or observation*).mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword]	25	Advanced
11	9 or 10	143	Advanced
12	remove duplicates from 11	140	Advanced
13	limit 12 to yr="1993-2015"	140	

Manually excluded studies on COPD patients

Web of Science

TS=(beclomethasone OR triamcinolone* OR flunisolide OR budesonide OR fluticasone OR mometasone OR ciclesonide OR (inhal* OR ics) SAME (corticosteroid* OR steroid OR glucocorticoid*)) AND TS=(trial* OR random* OR cohort* OR prospective* OR retrospective* OR observation* OR "case control*" OR study OR studies) AND TS=(asthma* OR copd OR "chronic obstructive" OR pneumoni*) NOT TI=(child* OR baby OR babies OR infant* OR newborn OR neonat* OR child* OR pediatr* OR paediatr* OR adolescen* OR teen*) 292

1993-2015

Scopus

TITLE-ABS-KEY ((beclomethasone OR triamcinolone* OR flunisolide OR budesonide OR fluticasone OR mometasone OR ciclesonide OR (inhal* W/5 (corticosteroid* OR ics OR steroid* OR glucocorticoid*)))) AND TITLE-ABS-KEY ((trial* OR random* OR cohort* OR prospective* OR retrospective* OR observation*) AND (asthma* OR copd OR "chronic obstructive") AND pneumoni*) AND NOT TITLE-ABS-KEY ((child* OR baby OR babies OR infant* OR newborn OR neonat* OR child* OR pediatr* OR paediatr* OR adolescen* OR teen*)) AND PUBYEAR > 1992 483

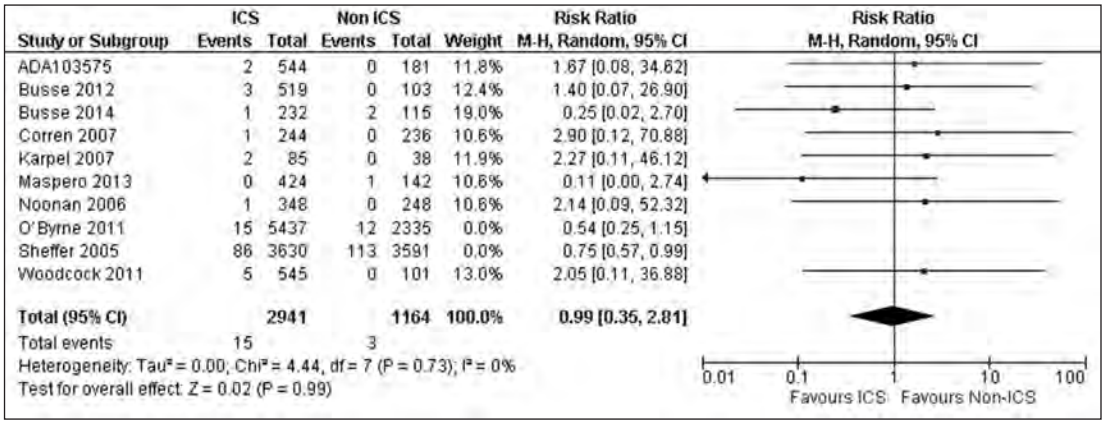

Figure S2 Sensitivity analysis for RCTs: Without Sheffer 2005 and O'Byrne 2011 studies data.

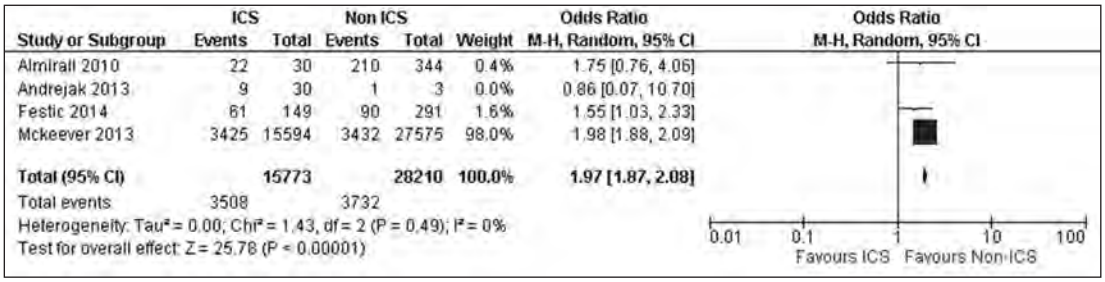

Figure S3 Sensitivity analyses for observational studies: Without Andrejak 2013 (NTM) study data.

Quality assessment scale for cohort studies

Note: A study can be awarded a maximum of one star for each numbered item within the Selection and Exposure categories. A maximum of two stars can be given for Comparability.

Selection

1) Representativeness of the exposed cohort
   A. Truly representative of patients with asthma on ICS in the community ⁎
   B. Somewhat representative of patients with asthma on ICS in the community⁎
   C. Selected group of participants
   D. No description of the derivation of the cohort
2) Selection of the non-exposed cohort
   A. Drawn from the same community as the exposed cohort ⁎
   B. Drawn from a different source
   C. No description of the derivation of the non-exposed cohort
3) Ascertainment of exposure (ICS)
   A. Prescription, medical records ⁎
   B. Self-report
   C. No description
4) Demonstration that outcome of interest (pneumonia) was not present at start of study
   A. Yes ⁎
   B. No

Comparability

5) Comparability of cohorts on the basis of the design or analysis: (a maximum of 2 stars can be allotted)
   A. Adjusted analysis (age = ⁎, other adjustments also = ⁎, i.e. Demographics, comorbidities, medications etc.)
   B. Unadjusted

Note: If the relative risk for the exposure of interest is adjusted for the confounders listed, then the groups will be considered to be comparable on each variable used in the adjustment.

Outcome

6) Assessment of outcome (pneumonia)
   A. Radiographic plus clinical diagnosis⁻ ⁎
   B. Clinical diagnosis only ⁎
   C. No description

   Assessment of outcome (pneumonia-related mortality, deaths in those with pneumonia)
   A. Reported ⁎
   B. Not clear

   Assessment of outcome (overall mortality, all deaths)
   A. Reported ⁎
   B. Not clear
7) Was follow-up long enough for outcomes to occur
   A. Yes (≥ 30 days or hospitalization for pneumonia) ⁎
   B. No
8) Adequacy of follow up of cohorts:
   A. Adequate follow up: >90% of subjects accounted for ⁎
   B. Acceptable follow up: >50% of subjects accounted for and unlikely to introduce bias or described ⁎

C. Follow up rate at the end of the study was < 50% and no description of those lost
D. No description

Quality assessment scale for case-control studies

Note: A study can be awarded a maximum of one star for each numbered item within the Selection and Exposure categories. A maximum of two stars can be given for Comparability.

Selection
1) Is the case definition adequate (pneumonia)?
   A. Clinical diagnosis ⁎
   B. No reference
2) Representativeness of the cases
   A. All eligible cases over a defined period of time/catchment area ⁎
   B. Appropriate sample of cases (random sample)
   C. Not stated
3) Selection of controls
   A. Same population as above (same community) ⁎
   B. Hospital controls
   C. No description
4) Definition of Controls
   A. No current (recent) ICS use ⁎
   B. Not stated

Comparability
5) Comparability of cases and controls on the basis of the design or analysis
   A. Study controls for age = ⁎
   B. Study controls for other factors = ⁎, i.e. severity, comorbidities, etc.)

Exposure
6) Ascertainment of exposure (ICS)
   A. Secure record (prescription, medical chart etc.)¯ ⁎
   B. No description or not as above
7) Same method of ascertainment for cases and controls
   A. Yes ⁎
   B. No
8) Non-Response rate
   A. Same no consent rate (refusal) for both/all groups ⁎
   B. Different no consent (refusal) rate non respondents described

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Review article

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Medication in the elderly - considerations and therapy prescription guidelines

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The aim of this study was to integrate and present pertinent findings from the literature dealing with the treatment of the elderly within a primary care setting. Medical care for the elderly is an integral part of a general practitioner's (GPs) everyday work and is challenging for many reasons. Older people often experience multiple chronic diseases concurrently (comorbidity, multimorbidity) and they often have deteriorated organ function and decreased physiological reserves due to the natural aging process. The choice of appropriate medication for each particular disease is a complex process and can cause "therapeutic confusion", especially among younger GPs in the field. Elderly people are prone to develop adverse side-effects to usual dosages of medications and the side-effects are even 7 times more frequent in elderly than in younger patients. Moreover, in therapy for elder patients, a responsible clinician always needs to think about potential drug to drug interactions and possible compromised pharmacokinetic dynamics in the aging body. Professional geriatric societies in many countries (USA, Germany, UK) have developed lists of potentially inappropriate medications for the elderly, and they update them systematically. Lists such as The Beers Criteria list and STOPP/START criteria should always be consulted when administering therapy to elderly patients. In this paper we emphasized the importance of medication lists as an important practical support in a GP's everyday work. Implementation of such therapeutic aids reduces the possibility of medical error and minimizes the chance of an inappropriate prescription for this vulnerable population stratum. **Conclusion**. When prescribing drugs for the elderly, GPs should take into account the specificities of the elderly, their biological and chronological framework and should always apply the principles of rational, conservative and evidence-based pharmacotherapy.

Key words: Elderly ▪ Rational prescription ▪ General practitioner ▪ Family medicine.

Introduction

Treatment of elderly patients exhibits special characteristics and is often a sensitive process within the scope of a general practitioner's work. Elderly patients present with more comorbidities, they often suffer from multiple disease conditions (multimorbidity), they commonly use more than a few medications (polypharmacy) and they have physiologically deteriorated organ function due to the natural process of aging. Quite often, it is a real challenge to administer proper therapy in elderly patients where there is significant potential for developing side-effects due to chronic use of drugs that can elicit strong systemic interactions.

Moreover, elderly patients are more prone to develop side-effects due to therapy, in comparison to younger adults. For example, use of antipsychotic medications among the elderly can induce severe anticholinergic reactions, Parkinsonian events, tardive dyskinesia, orthostatic hypertension, cardiac conduction disturbances, reduced bone mineral density, sedation and cognitive dysfunction (1). Likewise, elderly patients over the age of 70 are 3.5 times more likely than younger individuals to be admitted to hospital due to adverse drug reactions associated with psychotropic medications (2).

The aim of this article was to review recent literature on prescribing for the elderly.

Characteristics of elderly patients

According to the population census of 2001, 15.6% of the Croatian population was 65 years of age or older, and this population grew to 17.7% in the most recent census from 2011 (3). This trend places Croatia among the countries with a "very old population", according to criteria of the United Nations (UN) and the World Health Organization (WHO). The average life expectancy in Croatia is 71.1 years for men and 78.1 for women. Projections of the WHO are that in 2050 more than 25%, (even > 30%) of the population in Croatia will be aged 65 and over, with all the social, health and economic consequences of this process for the family and society as a whole (4). The elderly often suffer from chronic diseases and have multiple diseases at the same time (multimorbidity, comorbidity), which then often lead to polymedication, and sometimes polypharmacy (5). Polymedication is defined by some authorities as the use of more than four drugs simultaneously and is common in the treatment of elderly patients (6). There is a "thin line" that divides polymedication and polypharmacy while the latter involves inappropriate and purposeless prescribing of a number of drugs, that are not clinically indicated, to the same patient (7). These definitions have not yet been internationally standardized and the actual differentiation point that would define polypharmacy or polymedication is still an open question. With the increase in the number of prescribed medications, the risk of side effects and interactions dramatically increases. A study by Steinman et al. found that use of one or more inappropriate medications was documented in 65% of patients while 37% patients were taking medication in violation of the Beers drugs-to-avoid criteria, while as many as 57% patients took medications that were ineffective, not indicated or duplicative. In summary, inappropriate medication and overuse were common in elder people taking five or more medications, and this was present in more than 40% of patients (8). Likewise, the number of drug-related problems increased linearly with the increasing number of drugs used by patients in the study by Viktil et al. (9).

Altered and diminished physiological functions among elderly

When prescribing medications to the elderly, a responsible clinician needs to bear in mind that the pharmacodynamic (what the drug does to body physiologically) and pharmacokinetic (absorption, distribution, metabolism, excretion – what the body does to a drug) profiles are different among the elderly, in comparison to younger patients. This mostly occurs due to the natural aging process and it can be significantly altered in cases of various comorbidities and pathologies that increase with age. Absorption and distribution are reduced due to the reduction of total body water (10-15%) and serum albumins, as the major "carriers" of many drugs, decrease in the elderly by about 1/3 of the total concentration. The degradation and biotransformation of drugs in the liver

is also slower among the elderly because of the significant changes in liver physiology and functionality that are associated with aging. It has been proven that liver volume and blood flow decline with age in humans, and the clearance of drugs is diminished, particularly of those drugs that are oxidized by the microsomal cytochrome P450-dependent mono-oxygenase system (10). Moreover, the aging liver undergoes various histological changes, such as the increase of amounts of lipofuscin and shifts in the expression of various proteins that are synthesized by the liver (11). In addition to reduced hepatic clearance associated with age, kidney function also decreases due to a gradual reduction in the glomerular filtration rate (GFR) and the decrease in functional renal reserves (12). Many additional factors, such as the long-term attachment to bed, dehydration, congestive heart failure, and muscle atrophy, can significantly alter the pharmacokinetics with increasing age (13). Furthermore, the concurrent usage of multiple medications for different chronic conditions can precipitate significant drug interactions and may lead to drastic changes in the pharmacokinetic properties of medication administered (14). Non-compliance to drug therapy may be due to visual impairment, weakened motor skills and cognitive problems in the elderly, especially if a patient is taking anxiolytics or anticholinergics (15). Moreover, taking drugs without the assistance and/or control of family members may result in overdose and potentially lethal poisoning (16). Buying drugs over the counter (OTC drugs) as an addition to prescription drugs is also a source of potential danger due to adverse interactions (17). Some of the classic interactions are, for example: *Hypericum perforatum* with digitalis glycosides; Ginkgo biloba with acetylsalicylic acid (ASA); multivitamin preparations that contain vitamin K and coumarin anticoagulants or St. John's wort and concomitant use of antidepressants (18). From the most commonly used drugs among the elderly, the most common side-effects are well known in five classes of drugs: diuretics, digitalis glycosides, anti-depressants, analgesics and anti-hypertensive agents. The decisive role and full responsibility in prescribing for the elderly (which is not an easy task by any means) should be inherent to the family doctor's (GP) function. The GP alone has complete insight into all recommendations on medication for his elderly patient, which are given by different clinical specialists. The aim of this review is to examine the principles of rational and meaningful prescription, point out the specificities and offer some practical guidelines in medication prescription for the elderly in a GP's daily care.

Selection criteria

We reviewed the medical database Medline/PubMed and Google Scholar, using the 4 MESH keywords: elderly, prescription, general practice and family medicine. In respect to PubMed, the search yielded a total of 1597 articles and this was filtered down to 181 articles when those published within the last 5 years were selected. When searching Google Scholar, we chose those articles that had a high citation index and a substantial impact in the field of general/family medicine and that were ranked by their significance and total citations. By combining these two criteria, we not only aimed to select those articles that were recent and contemporary, but also to include those that had pertinent impact and relevancy in the field of clinical family medicine and the general practitioner's arena, regardless of publication date.

Discussion

When prescribing for the elderly, it is recommended to respect the paradigm of conservative prescribing, and these basic principles

are presented by Schiff and associates in his principles of conservative pharmacotherapy (19). In this light, we will use Schiff's principles as the foundation to further elaborate useful practical guidelines, with additional relevant sources from the literature. Before prescribing, a responsible clinician should:

Consider non-pharmacological treatment alternatives for the disease/condition

Many diseases are caused by unhealthy and sedentary lifestyles that are intrinsically marked by low levels of physical activity (20). Instead of "over-medicalization", counseling on healthy eating, emphasizing the importance of physical activity and smoking cessation recommendations should be propagated by GP's to the highest possible extent. A recent study showed that physicians discuss the risks of smoking with their patients, however, practical cessation support is often inadequate (21). In some cases, instead of the conservative approach, surgery should be advised in instances where a pharmacological approach could impose a significant burden on the patient (22). Likewise, a GP needs to be aware that important life changes and decisions in the life of the elderly patient may be achieved if the patient is in a stable psychological state, without the depressive symptomatology that is often encountered among elderly (23). In that regard, psychotherapy can have beneficial effects on elderly patients and may reduce the unnecessary burden of psychotropic drugs. An IMPACT study conducted in the primary care setting showed that psychotherapy, alongside pharmacotherapy, managed to achieve lower levels of depression, better physical functioning and an enhanced quality of life among depressed older adults (24). Similarly, application of local heat and cold pads can reduce pain and help to achieve analgesia, without excessive utilization of non-steroidal anti-inflammatory (NSAID) drugs for arthritic conditions (25) . A TONE study showed that reduced sodium intake and weight loss, achieved through physical exercise, constitute an effective and safe non-pharmacologic therapy for hypertension in older persons (26). This can significantly reduce the antihypertensive medication burden. These are only some of the examples that show how non-pharmacologic interventions can yield substantial benefits among the elderly, and should not be underestimated.

Consider the potential causes of the disease, do not only cure the symptoms

Dyslipidemia among the elderly can be a symptom of unrecognized and therefore, untreated hypothyroidism, osteoporosis can be the underlying cause of arthralgia, while erectile dysfunction can be a physical manifestation of underlying psychological or psychiatric entities, such as performance anxiety, stress or mental disorders. Therefore, we should always search for the principal, underlying cause of the basic disease, and we should not only cure symptoms (27).

Use a preventive approach and not only treat an already developed and advanced disease

Preventive activities are an integral part of the daily work of family doctors. Combating unhealthy lifestyles, such as smoking, inadequate diet, and obesity, with all their possible consequences, and prevention of risky behavior in the "long run" are much more effective. The treatment is much more expensive and less effective (28).

Use the function of time as a diagnostic tool in your clinical judgment

In the prodromal or early febrile phase of some infectious diseases, it is not always simple to evaluate whether the symptoms and signs are caused by viruses or bacteria. In some cases it is appropriate to use the function of time as an aid in diagnosing the

etiology of a disease. This has some practical implications - for example, we might delay the institution of antimicrobial drug therapy by using the technique of *"watchful waiting"*, also known as *"watch and wait"* or WAW, where applicable. This approach could be utilized in situations where there is a high degree of certainty present that the disease could self-resolve, or in situations in which the risks of therapeutic modality might outweigh the potential benefits. Such situations are often encountered by GPs in patients that present with acute otitis media with effusion, inguinal hernia, pneumonia, rhino sinusitis or benign prostatic hyperplasia (BPH) (29-31).

Use a relatively small number of drugs, know them well and use them appropriately

A responsible general practitioner should have a solid knowledge of the most common and important groups of drugs that he/she is prescribing, including indications and contraindications for their use, the most common side-effects and pharmacological interactions. This approach increases the quality of prescriptions and reduces the error. In a recent study, in which polypharmacy was defined as the use of six to nine drugs at the same time in one patient there was a significant association between polypharmacy status and mortality (32). STOPP/START criteria, that measure potential inappropriate prescribing, have a significant correlation with medication-related hospital admissions in older patients (33). A similar study showed that polypharmacy correlates with an increased risk of hip fracture in the elderly (34). Therefore, it is clear that in some instances *"more"* is not always synonymous with *"better"*.

Avoid switching drugs or abrupt withdrawal

Reasons for switching existing medication to new forms should be well-founded. The rule is that any modification of treatment and / or replacement of one drug with another must be justified and clearly reasoned. For example, institution of a new diuretic or a vasodilatatory drug in the treatment plan can induce orthostatic hypotension among elderly patients (35). Likewise, swift removal of beta-blockers from the line of therapy can induce reflexive tachycardia and may decompensate a patient with heart disease (36). In a similar way, sudden withdrawal of an anti-Parkinsonian drug therapy among the elderly may result in neuroleptic malignant syndrome (37).

Start treatment with a single drug, whenever possible

Such prescribing ensures the easy identification of potential side-effects. The less medication we use, the fewer difficulties we have in identifying the causal agent that induced the side-effects. For example, antihypertensive therapy needs to be based on evidence–based medicine (EBM), and administration of a new drug should be clinically justified only in cases in which the therapeutic goals (for example, the desired level of arterial blood pressure) have not been met (38).

Think about the possible side-effects, anticipate them and inform patients about them

Lack of knowledge about side-effects can result in what is known as "*prescription cascade*" - a physician does not properly recognize the side effects of the drug and wrongly misinterprets them as a new entity or a disease (39). Therefore, he/she then prescribes a new drug for the latest condition, which often masks and/or complicates the clinical presentation and so on. It is of cardinal importance for the conscientious general practitioner always to assess any new symptomatology that occurs among patients that

are under therapy, because these undesired symptoms may be the consequence of the patient's drug treatment (40). This cascade can be stopped by de-prescribing. De-prescribing is a process that entails reduction or complete withdrawal of a drug that might cause side-effects and undesired symptoms (41). It has been proven that systematic and careful de-prescribing can improve the quality of life and cognitive function, and can foster better therapy compliance rates among the elderly (42). While the consequences of de-prescribing are fairly rare, the dangers of adverse drug withdrawal events still persist (36). In this respect, it is important to have in mind that discontinuation of certain drugs from the line of therapy requires gradual and titrated withdrawal, and they should not be removed abruptly and in a short period of time, due to the potential risks.

When a new drug appears, do not rush to use it

Information about new drugs should be provided using relevant EBM sources which are impartial and not influenced by the pharmaceutical industry. It takes 5-10 years of use to recognize all the side-effects of a drug. Physicians can often "fall into the trap" of prescribing a new drug because they are pressurised by the pharmaceutical representative, firms, hospital consultants or the patients themselves. The decision to initiate a new drug is often heavily influenced by "who says what" (43). Moreover, an early good experience of using a new drug can strongly influence future use (44). A study by Adair & Holmgren showed that drug samples received from pharmaceutical companies can influence prescribing behaviors among residents (45). It is important to build awareness about such dynamics and avoid them whenever possible.

When prescribing, one should not be exclusively guided by the wishes and desires of the patient

A relevant study found that a physician's behavior in terms of prescribing medication is most strongly associated with the perceived medical need of the patient, which strongly confounded other predictors (46). Patients' requests for medicines are a powerful driver of prescribing decisions. In many cases, physicians prescribe the requested medication although they may be ambivalent about the drug in question (47). This should be avoided in daily practice whenever possible. A responsible GP needs to be observant and open towards the patient's reported needs and preferences, however, the decision about treatment should primarily be based on EBM and good clinical practice.

When the therapeutic effect of the drug fails, consider that non-compliance by the patient could be the reason for the "ineffectiveness", not the drug itself

Low patient compliance can significantly undermine treatment effectiveness as has been proven in multiple studies (48). The health outcomes differ by 26% between groups of patients who had high compliance to therapy vs. those who exhibited poor compliance (49). Every GP should focus his/her efforts on trying to increase compliance to short-term treatments, such as counseling about the importance of compliance, written instructions about taking medicines and reminder packaging, among many others (50).

The Beers Criteria (List) and STOPP/START Criteria – an important foundation for rational pharmacotherapy among the elderly

By respecting all the principles discussed above, excessive prescribing could be con-

verted into a meaningful and rational prescription process. In 1991, Dr. Mark Beers, through a consensus panel of experts using the Delphi method, developed criteria of inappropriate prescribing for the elderly, named after him - The Beers Criteria for Potentially Inappropriate Medication Use in Older Adults, commonly known as the Beers List. This list contains lists of medications that could pose a higher risk than potential benefit for people aged 65 and older (51). It is important to highlight that the Beers List is not a substitute for professional judgment in prescribing decisions for the elderly, but it is intended to serve as guidance material for clinicians. The American Geriatrics Society (AGS) applied these criteria and revised them according to the list of drugs available in the US, with the principal intention of improving prescription for the elderly, reducing the incidence of unwanted side-effects and reducing unnecessary costs of medication (52). According to the AGS Beers' criteria a total of 53 drugs and/or drug groups are stratified in three categories: I. "*unsuitable for use in the elderly*", II. "*unsuitable for use in the elderly in certain diseases and conditions*", III. "*may be used with caution*". A similar process was applied to the list of drugs available on the German market and, thereby the PRISCUS list was created and is nowadays used in Germany as an integral part of geriatric pharmacotherapy (53).

The importance of inappropriate prescribing as a phenomenon has been recognized by Irish researchers, who have created and validated an assessment instrument – the "Screening Tool in Older Persons for Potentially Inappropriate Prescriptions and the Screening Tool to Alert Doctors to the Right Treatment", commonly known as the STOPP/START criteria (54). This study was conducted at University Hospital Cork and it involved the population of elderly patients referred by their family for acute illness conditions. By using the STOPP criteria, inappropriate medication was detected as follows: 25% of people were receiving one, 7% received two and 2% three inappropriate drugs. The most common errors were application of long-acting benzodiazepines and/or tricyclic antidepressants in patients with clear contraindications, as well as the use of drugs that increase the incidence of falls in patients who are prone to them. In addition, a common error was "duplication" - two prescriptions of NSAIDs, ACE inhibitors, SSRIs or two anti-platelet drugs for the same patient (55). The question is: will regular updating of the list of medicines for the elderly in these countries actually lead to more rational prescribing, fewer prescribing errors, a lower incidence of unwanted side-effects and reducing health care costs in the future? A revised version of the STOPP/START criteria was published in March 2015 and these guidelines are up-to-date with literature reviews and consensus validation by a European panel of experts (56). To sum up, the Beers List and STOPP/START criteria are often used complementarily to guide clinicians in the safe drug prescribing in older adults, and should always be consulted (57).

Final thoughts

GPs play a key role in preventing unnecessary polypharmacy in elderly. Their role is to weigh and assess each treatment recommendation given by clinical specialists. By knowing their elderly patients, their diseases, conditions and family/social situations, GPs are in an ideal position to make an appropriate selection of a drug for an individual patient. In this process, the GP should determine "priority" diseases, always taking into account the possible side-effects and interactions. The Brown Bag Medication Review is a six-month review method that takes into account all medications taken, and it has proved very effective in reducing polymedication. It implies that the patient, once

in each six-month period, brings all medications taken regularly "in a brown bag" to the office of their GP, including the medication prescribed by the doctor and all others (OTC medicines, herbal remedies etc.). In this way, patients can receive the maximum benefit from their medication and reduce drug wastage (58). Before prescribing a drug to a patient, the GP should always answer several key questions to determine if the treatment regimen is justified (Box 1).

Box 1 The questions that GPs should answer themselves before prescribing any drug to an elderly person within the primary care setting

1. Is the drug really necessary?
2. To which pharmacodynamics group does the drug belong and what is the mechanism of its action?
3. What do I want to achieve with this drug?
4. How do I evaluate the effectiveness of this drug?
5. What dosage shall I give and for how long?
6. Did I choose the simplest therapeutic scheme?
7. Did I assess both the biological and chronological age of the patient before prescribing the drug (because these are not always consistent)?
8. Can the existing disease, other medications, or the age of the patient affect the absorption, metabolism or excretion of a drug?
9. What possible side effects should I expect?
10. Will the patient take the medicine? Could motor weakness, visual, cognitive or other impairments compromise compliance in taking the drug? Is the patient capable of taking medicine alone, or should he/she be helped and/or supervised by others?
11. Do I need to provide further clarification about the mode of taking, actions and side-effects of the drug to the patient or to their family member(s)? Should I write down a scheme of administration (dosage, schedule, depending on meals)?

Conclusion

When prescribing drugs for elderly, GPs should always take into account the peculiarities of the elderly and their biological/chronological age. A responsible clinician should always apply the principles of rational pharmacotherapy and conservative prescription, while avoiding the trap of fashionable and harmful prescribing that is not evidence-based.

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Historical article

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Dr. Stanko Sielski (1891–1958): Physician, scientist, humanist

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This work presents the results of research into the life and work of Dr. Stanko Sielski, related to his professional, scientific and humanitarian work. He was born in Gračanica, Bosnia and Herzegovina (BH) in1891, to a family of Polish origins. He attended high school in Travnik and completed his studies of medicine in Vienna in 1919. During the First World War he served on the frontlines with the Austro-Hungarian army. He began his service as a doctor in Konjic, Prozor and Glamoč, and then worked in Varcar Vakuf, Zenica, Travnik, Bihać, Banja Luka, Sarajevo and Tuzla. At that time in BH living conditions were very bad, the level of education of the people insufficient, there were many epidemics of infectious diseases, and the mortality of the population was high. Dr. Stanko Sielski made a significant contribution to treating the sick, preventing various diseases and the health education of the people. In the realm of the history of medicine in BA, he researched the life and work of doctors from previous generations, the work of medical institutions, old medical manuscripts written in Arabic, Persian and Turkish, folk beliefs about the origins and treatment of a variety of illnesses, and the role of herbal medicine and amulets in treating the sick. In addition, he undertook research in the fields of archaeology, ethnology and sociology. He published the results of his research in scholarly journals. In the Second World War he saved the lives of many Jewish doctors and their families from persecution in concentration camps, and as a result in 2014 he was posthumously declared "Righteous Among the Nations". **Conclusion**. Dr. Stanko Sielski, alongside his work as a doctor, was also involved in a variety of scientific research and publication work, which contributed to the preservation and a better understanding of the material and spiritual heritage of BH.

Key words: Stanko Sielski ▪ History of medicine ▪ Archaeology ▪ Ethnology ▪ Bosnia and Herzegovina.

Introduction

It will soon be 125 years since the birth of Dr. Stanko Sielski, the physician, humanist and scientist, who is worthy of our interest, not only in the field of medicine, but also in the fields of ethnology, ethnography, archaeology and sociology. He was a man, led by his feelings, who helped people in various ways, regardless of their social status, ethnic and religious affiliation, and sometimes even regardless of the current moral values of the environment he was working in.

The majority of the working life of Dr. Stanko Sielski was marked by the social and economic consequences of the First and Second World Wars. At that time in Bosnia and Herzegovina (BH) the living conditions were very bad, the level of education of the people poor, the hygiene of the population,

especially in the villages, was at a low level, infectious diseases frequently took on the characteristics of epidemics, and the resulting mortality of the population, especially children, was high. The life of society, in many aspects, was based on "customs", which were deemed to be the best and only way to behave, and quackery (folk medicine) was deeply rooted.

Although he was a physician, who made a significant contribution to improving public health in BH in his time, a humanist and a fruitful cultural and scientific worker, no one has yet undertaken a thorough analysis, to research his life and work systematically, and present it to the wider cultural public of BH, but also the public of the wider environment. As a result his broad and varied opus of great importance for his own country, has mainly remained unknown, or is only mentioned in fragments.

This paper presents the results of research into the life and work of Dr. Stanko Sielski, relating to his professional, scientific and humanitarian work.

Childhood and education

Stanko Sielski was born in Gračanica, BH, in 1891, to a family of Polish origin, who moved to Bosnia from Tarnopol (Galica) at the end of the 19th century. His father Stanislav was a geometer in civil service, and his mother Marija, nee Waldher, was a homemaker. His father was frequently sent to work in different places in Bosnia, so Stanko spent his childhood in Gračanica, Zenica and Travnik. He attended high school in Travnik, and after graduating in 1910 he enrolled to study medicine in Vienna, where at the same time he studied painting and the history of art for two years (1). During his studies, he served as a soldier on the frontline in the Austro-Hungarian army during the First World War, and later as a medic, working as a doctor. After the end of the war in 1918 he was discharged from the army with the rank of Lieutenant. He then continued his study of medicine in Vienna and graduated on 16th June 1919 (1).

Professional work

After graduating from the Medical Faculty, Dr. Stanko Sielski remained in Vienna until 1st April 1919, when he worked as a Lieutenant in the Military Mission Service in the Kingdom of Serbs, Croats and Slovenes (1). He began his state service as a doctor on 16th November 1919 in the District Administration of Konjic, Prozor and Glamoč, where he worked until 30th November 1920, as a honorary doctor, specialized in epidemiology combating typhoid and smallpox. After that, until 19th February 1924, he worked as a district doctor in Varcar Vakuf (Mrkonjić Grad), then he was appointed to Zenica in the same capacity until 12th November 1924, after which he continued to work in Travnik as a district doctor, the local sanitary officer and health advisor, until 3rd November 1931. By a decree of the Royal Ministry of Public Health of the Kingdom of Yugoslavia of 3rd November 1931, Dr. Stanko Sielski was transferred to Bihać, where up to 19th July 1941 he worked as a health advisor, and senior advisor and manager of the Public Health Centre (1).

In all these places, the sanitary and epidemiological situation was very severe, infectious diseases usually took on the characteristics of epidemics, and the hygiene of the population, especially in the villages, was at a very low level. As a physician Dr. Stanko Sielski made a very significant contribution in treating the sick, but also in the field of combating infectious diseases, parasite and fungal infections and pediculosis, and in providing health education for the people. His special contribution was in treating and preventing endemic syphilis in Bihać (2).

Dr. Stanko Sielski worked in Banja Luka during the Second World War (19.7.1941-25.8.1944). He was the head of the Institute for Combating Endemic Syphilis (the Institute) (Pictures 1, 2), which was founded by the Independent State of Croatia, in order to root out this disease once and for all, as quickly as possible from Bosnian villages (3).

Picture 1 The building of the Institute for combating endemic syphilis in Banja Luka 1941-1945.

Picture 2 Dr. Stanko Sielski at the time when he was working at the Institute for combating endemic syphilis in Banja Luka.

The seat of the Institute was in Banja Luka, but the actual work took place through the health institutes of that time, the public health centres, and other health institutions in BH, sometimes also improvised in-field clinics in the remote Bosnian villages (Picture 3) (4).

Picture 3 An improvised in-field clinic of the Institute for combating endemic syphilis.

Doctors from the Institute were mainly Jews from Croatia and BH, who had already been, or should have been deported to labor camps or death camps. Since the doctors were mainly accommodated in small Bosnian towns where there were no permanent or occasional doctors, they treated the population not only for syphilis but also for other illnesses.

Dr. Stanko Sielski worked in Banja Luka in the relatively short, but extremely complex and difficult period of the war. He also held the function of a managing civil servant in a state which from its very outset, following the example of the Third Reich, accepted and made law the National Socialist interpretation of race, which was mainly aimed against Jews and Roma, but also all those who did not agree with Ustasha politics (5). It was precisely in these circumstances that his altruistic character reached its full expression. He supported the People's Liberation Movement with hygienic and medical materials, and he enabled some Home Guard (Domobran) soldiers to transfer to Partisan units. His role in saving Jewish doctors, the employees of the Institute, from persecution in concentration camps was extremely im-

portant. According to the oral and written statements given to the writer of this text by the last living doctor from the Institute, Dr. Teodor Grüner from Zagreb, Dr. Stanko Sielski saved him and many other Jewish doctors and their families, whereby he also risked his own life (6). This testimony, along with the proposal (7) by the author of this text, was a crucial document for the posthumous declaration of Stanko Sielski as *Righteous among the Nations* at the end of 2014. This prestigious award by the State of Israel is awarded to non-Jews who exposed their own lives to danger during the Holocaust to save Jews from persecution by the Nazis and their collaborators. However, when this decision was rendered an unforgivable error was made, since Dr. Stanko Sielski was designated as Righteous of the State of Croatia, whereby BH was overlooked, that is the state in which he was born, where he was educated and in which he worked productively to the end of his life.

On 31st July 1944, Dr. Stanko Sielski was transferred to service in the Ministry of Public Education of the Independent State of Croatia, that is to the Faculty of Medicine of the Croatian University in Sarajevo (8). While he was still working in Banja Luka at the beginning of 1944, he was elected to be private assistant professor of the Chair of History of Medicine of the Zagreb Faculty of Medicine, in the subject of Folk Medicine (9), and soon after that he was also elected to be regular professor in the subject of History of Medicine at the Faculty of Medicine in Sarajevo (10) and the first dean of that faculty (11). He worked as the dean of the Faculty of Medicine in Sarajevo from 1st August 1944 to 13th May 1945. After the liberation of Sarajevo in April 1945 and the hand over of the Faculty to the new authorities on 13th May 1945, he was sent to work on the District People's Committee in Banja Luka (12). In June of the same year, he was sent to Kozara as part of a hygiene and epidemilogical team of the First Army, in order to help combat typhus (13). He stayed in Banja Luka until 2nd March 1946 when he was transferred to Tuzla (14).

In Tuzla he worked as the head of the health and epidemiology centre, later renamed the Institute of Hygiene, right up until his death on 31st October 1958. This was the period immediately after the Second World War, and the scope of work of that health institution covered the region of north-east BH. The social and economic situation in the state was difficult, the population were impoverished and exhausted after four years of war; hygiene, especially in village areas, was at a very low level, and epidemics of various infectious diseases were frequent, which contributed to the fact that mortality, especially amongst children, was high. Dr. Stanko Sielski, who was sometimes the only physician at such an important and significant health institution, made an important contribution to improving health in that area together with his co-workers. He died at the age of 68 in Zagreb, where he was buried in Mirogoj cemetery.

The scientific and publication work

Dr. Stanko Sielski was involved in scientific research work throughout his working life. Although he worked mainly in small towns and villages, this did not prevent him, far from major institutions, from taking a lively interest in the medical profession, and precisely in those small Bosnian towns, and he found subjects to satisfy his interest in research, subjects which were sometimes very close to him, and sometimes in complete contrast to his profession.

In the realm of the history of medicine in BH, he researched the life and work of doctors from previous generations, (15), the work of medical institutions, (16), old medical manuscripts written in Arabic, Persian and Turkish, (17), folk beliefs about the ori-

gins and treatment of a variety of illnesses, (18-22) and the role of amulets and folk medicine books in treating the sick (23, 24).

Of particular benefit for archaeological, ethnological and ethnographic science in BH was the constant contact and collaboration between Dr. Stanko Sielski and the museums in the places where he worked (25-28), his references to new archaeological finds and his assistance to museum experts during their in-field work (29, 30), as well as his research in the area around Travnik and Žepče (31) and Bihać (32, 33). There are many written documents and other exhibits in museums in BH and Croatia bearing testimony to this (34-37). In the final years of his life, Dr. Stanko Sielski was an associate of the Yugoslav Lexicographical Institute in Zagreb, in writing the first volume of the Medical Encyclopaedia, which was published in 1958 (38).

By careful research, I have found evidence to establish that Dr. Stanko Sielski published 20 papers: 16 from the realm of medicine, two from archaeology, and one each from the fields of ethnography and sociology (39). However, this does not mean that there are not other works by him for which I have not yet been able to find documentation. The lectures are also not listed here which Dr. Stanko Sielski presented at various professional congresses, symposia and other conferences, because I was not able to find documentation for them, although I learned about them from a variety of reports and the daily press.

The paper: "Brill's disease. IV. Study of 26 cases in Yugoslavia" of which he is one of the co-authors, was presented to the Epidemiology Section of the American Public Health Association at the Seventy-ninth Annual Meeting in San Francisco, California, on October 30th, 1951 and published in *The American Journal of Public Health* (40). It is so interesting that it deserves to have a separate article written and published about it. Otherwise, all his other articles, published in the Bosnian language, are worthy of attention, however, the best insight into his entire work in research is given by the articles "Dr. Justin Karlinski", "Old Turkish and Arabic Medical Manuscripts in Bosnia and Herzegovina", "Amulets", "Archaeological Finds in the Area of Travnik and Žepče" and "Our Village Golubić – a contribution to research into health in the village and life there", and they therefore deserve a special mention here.

The article: "Dr. Justin Karlinski", which Dr. Stanko Sielski published in 1953 in the journal, *Higijena: časopis za higijenu, mikrobiologiju, epidemiologiju i sanitarnu tehniku* (Hygiene, journal for hygiene, microbiology, epidemiology and sanitary technology) (15) was the result of the interest of Dr. Stanko Sielski in the life and work of that physician. That interest was awakened in him by articles written by Dr. Justin Karlinski, which Dr. Stanko Sielski read whilst leafing through a copy of the journal *Glasnik Zemaljskog muzeja u Sarajevu* (The Herald of the National Museum in Sarajevo), much earlier than the time when he decided to write about this famous doctor in BH. It is interesting how Dr. Stanko Sielski came upon the carefully preserved documentation of Dr. Justin Karlinski. After arriving in Tuzla, he met Mrs. Marcela Karlinski, the widow of Dr. Justin Karlinski and their daughter, Zosja, then Mrs. Opitz. From them he learned that Dr. Justin Karlinski, alongside his practice as a doctor, during his work in Bosnia had also been involved in scientific research work, and they had kept six hard covered volumes, comprising copies of his articles written in various languages and printed in various European journals, which formed both his private and official documentation. At his request, Mrs. Marcela Karlinski allowed Dr. Stanko Sielski to examine these documents, and he, after he had studied them in detail, wrote the article about his life and work. Dr. Justin Karlinski would have been completely

forgotten in BH, if Dr. Stanko Sielski had not written that article, which comprises 14 pages of densely typed text, with a picture of Dr. Justin Karlinski, and a list of his 80 publications written in the form of references. The text is a studious and well summarized analysis of the life and work of the interesting and unusual personality of Dr. Justin Karlinski. It is not necessary to go into more detail about this article here because that would be a summary of something that has already been summarized, but I warmly recommend it to those who are interested in how a highly educated physician and scholar lived and worked in BH at the end of the 19th and the beginning of the 20th century.

The article: "Old Turkish and Arabic Medical Manuscripts in Bosnia and Herzegovina", which Dr. Stanko Sielski published in the book *Iz Hrvatske medicinske prošlosti* (From the Medical History of Croatia) in 1954 (17), did not occur spontaneously, but was the fruit of his many years of systematically collecting and researching historical data, various documents, manuscripts, articles and books, which were used long ago in Bosnia as a written source of knowledge for treating the sick. The article comprises 16 pages of dense, type-written text and has four illustrations. At the end of the article there is a chapter that explains the foreign words used and a list of literature. Although the article is not systematized in the sense of having titles and sub-titles, it is written in an orderly manner with recognisable paragraphs, and the text itself forms a well connected whole, which is easy to read and interesting.

On the first page, in the first paragraph, Dr. Stanko Sielski indicates the motives and reasons for writing this piece. This is how he describes it: "Loyalty to this country, where I was born, and have lived and worked, dictated these lines to me, where I will describe briefly the lives of some old eastern physicians and their work, which I have found in Bosnia". Later, also on the first page, he writes about Bosnia: "There are few places in the world where contrasts have so obviously come into conflict. Through the ages fortified cities and towers have stood like wreaths around the borders of our republic. On this heroic battlefield and country, in which at one time everyone carried arms to be able to defend their golden freedom, our people fought and fell through the ages, and the innumerable memorial stones are the silent witnesses to plague, famine and war. The old memorials and walls are overgrown with brush and creeping vines, and the wind blows away the few reminders of those who worked, and as a result of historical events, ploughed hard furrows in the lives of their blood brothers".

At the end of the "introductory" part of the article, which comprises a text of more than three pages, Dr. Stanko Sielski writes that the purpose of the article was to present to our historians how oriental medical books were studied, revealing many writers, previously unknown to us, and including original Bosnian ideas, since, as he writes, the Bosnian scribes, at the very least, when they were copying the oriental manuscripts, added their own personal comments to them, their own experience, and their own methods of treatment.

Most of the remainder of the text relates to extensive or brief analyses of individual medical manuscripts found in BH. These analyses include brief or extensive biographical data on the authors of individual pieces, and there is also a detailed description of the work itself, in which he as a doctor emphasizes and comments on interesting chapters, and sometimes presents them verbatim in his text and links them to medical practice of his time.

Dr. Stanko Sielski pays particular attention to the text written in the margins, known as *marginalia*. This is to be expected from him, because the margins contained notes and comments by unknown local

scribes, who were to Dr. Stanko Sielski interesting, modest and extremely important characters. He frequently points out their Bosnian origins, which is also to be expected of him, because at all times he was primarily interested in Bosnia. In this way Dr. Stanko Sielski dealt with 7 medical works. At the end of the article he thanks his friend, Prof. Abdurahman Čokić from Tuzla, and Prof. Dr. Šakir Sikirić from Sarajevo, the well-known Bosnian Orientalist, for the assistance provided in translating those manuscripts.

In the article "Amulets" published in 1941, in the publication *Etnografska istraživanja i građa III* (Ethnographic Research and Materials, III) by the Croatian State Ethnographic Museum in Zagreb in 1941 (23) Dr. Stanko Sielski, as a proficient researcher, and a thorough and widely educated intellectual, describes various aspects of "various items" - from amulets and spells, to apotropaic scriptures, which helped people in various forms of trouble, or protected those who used them from different forms of evil, objects which he collected while he was working as a doctor, mainly from the Bosnian Frontier (*Bosanska krajina*), and he gave them or sold them to the Ethnographic Museum in Zagreb. One of those objects is shown in Picture 4.

Already at the beginning of this article, Dr. Stanko Sielski, with enviable interest, unravels and explains folk beliefs about objects with "talisman powers", telling how these objects had an important role in our folk medicine and beliefs. He attempts, on the basis of the knowledge he acquired during his many years of research, to categorize and define the differences between true amulets, amulets against spells, talismans and similar objects, stating thereby that it was difficult to differentiate them, or even impossible to define clear boundaries between them. He describes apotropaic scriptures, stating that they were adjusted to the religion of those wearing them, and how previously the custom had been that a hodja wouldisto write them for Christians or Christian priests for Muslims, but that had already become a rarity, because Catholic and Orthodox priests had stopped writing apotropaic scriptures long before, whilst hodjas still did so.

Picture 4 Enamluk, a container for texts from the Quran (*enam*) made from silver leaf, decorated with filigree, amethysts and coral (against spells). Pendants – more recent. Allegedly once the property of a captain from Tuzla in Travnik. Ethnographic Museum in Zagreb, inv. no. 13698.

With an enviable and undisputed feeling for research into these items with mysterious power, Dr. Stanko Sielski describes what apotropaic scriptures were for, what they looked like, their size and the material they were written on, the writing implements used and the way they were written, and the places on the body where they were worn, which often reveals the essence of the written text. They frequently included statements by those who wrote them, or the people who wore them. In this way, through 49 pictures, showing more than 100 items with accompanying texts, he describes the various items used by the people of the Bosnian Frontier for different types of problems, or which "defended" them from spells.

The article: "Archaeological Finds in the Area of Travnik and Žepče" was published

by Dr. Stanko Sielski in the *Glasnik Zemaljskog muzeja u Bosni i Herzegovina* (The Herald of the National Museum of Bosnia and Herzegovina) in 1931 (31). The article covers six pages, organized into six chapters, after which there is a summary in German. There is an Appendix to the article, consisting of 12 tables printed on a separate sheet of paper, marked in Roman numerals from 5 to 16, in which there are black and white sketches of items found, mainly life-size, numbered from 1 to 149, to which the author refers in the textual description. These are rare examples of the artistic work of Dr. Stanko Sielski, because his works of art have mostly not been preserved.

The first chapter: *A Neolithic Settlement in the Valley of the River Bila*, begins with a brief description of the site, its geographical position and a description of the research. The author goes on to state that flint knives, scrapers, arrows, stone axes, wedges, grinders and hammers fragments of clay pots with or without ornamentation were found there. On the basis of the items found, the author presumes: "our ancestors living on the banks of the River Bila easily met their everyday needs". He then states that the research was not systematic, that is that the dig was random, that the order of the layers was mainly doubled, and there follows a systematized description of the items found. At the end of this chapter, the author points out that amongst the bronze items he also found a brooch decorated with some form of flower and dots, which is similar to a brooch or pendant described by Kellner in his article "Remains of Roman Settlements on Ilidža", which was published in The Herald of the National Museum of Bosnia and Herzegovina, volume 5, 1897, on page 149, and he concludes that this brooch dates from the Roman era.

There follows a shorter chapter: *Bronze Finds in the Valley of the River Bila*, which describes the location of the site, and the findings of bronze jewellery, consisting of twisted necklaces with no ornamentation, two spiral bracelets, a spiral ring and a double clasp. The third chapter: *Bronze Finds in Brezovo Polje*, begins with a brief history of the discovery and a description of the location of the site of a bronze store, comprising 16 celts, 3 spears and one sickle, where all the items except the sickle have been well-preserved and covered in beautiful green patina. The textual description of these items is given in a clear and systematized table.

In the fourth chapter: *Bronze finds from Koričani*, there is a description of a well-preserved, ornamented bronze celt (an implement like a chisel or axe head), covered in green patina, which was found by a shepherd in the village of Koričani in the county of Jajce. This is followed by chapter five: *The find of coins of King Tomaš*, which describes the location, the circumstances of the discovery, the appearance and details on the head and tail sides of 13 coins, dating from the period of the reign of King Tomaš (1444-1461). In the description of the appearance of these coins, the description of a smaller circle is emphasized, which is found on both the head and tail sides, of which the author writes: "He has not noticed any earlier description of these on coins of this type, so he does not know whether the description had simply been omitted or other coins of this type do not have these circles, but this unusual feature needs to be noted since the circle was formed with unusual delicacy."

The final, sixth chapter: *Medieval finds from the Village of Zabilje in Travnik County*, gives a presentation of the discovery and description of two iron spurs and one iron knife in the "fort" above the village of Zabilje. The author points out that these items were found by the farm labourer Nezir Karahodža, when he was clearing the forest, in a barrow with human bones, and they were kept on the roof of a house. At the end of this short chapter, the author writes: "Knives like this have been found frequently

in pre-historic pile dwellings (Radimsky pile dwelling near Ripač, Journal, vol. V, page 41) as well as amongst Roman remains, but according to this find, it is probable that these are medieval items."

"Our Village Golubić – a contribution to research into health in the village and life there" is an article published in the journal *Socijalno medicinski pregled* (Social Medical Review) in 1939, which was published in Belgrade (33). It covers 22 pages in Cyrillic script, and is illustrated with black and white sketches, with a summary in German and no literature listed. In it, Dr. Stanko Sielski describes the social conditions in the village of Golubić, which is located near Bihać, and sometimes comments on their effect on the health of the population.

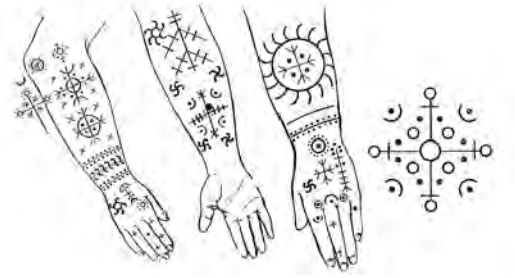
He writes of his choice of that village: "This village was chosen because it is only six kilometres from the health centre, that is from the centre of our work, so it is easily accessible. But it was also chosen because there are Orthodox and Catholic believers as well as Muslims living together there, so it is interesting not just from a geographical but also from an anthropological and ethnographic point of view". There follows a geographical and historical description of the village, then chapters on the population, the climate, the way the land was worked, fruit growing, trades, folk costumes, the furnishings of the houses, how rooms are decorated, types of houses, yards, barns, family life and customs, the social status of men and women, customs and diet.

It is important to point out that in the chapter entitled: *The Population,* alongside general information, the author gives specific results of anthropometric measurements made, the frequency of blood groups, eye colours, the shape of shoulder blades, and when menstruation begins and ends in the women. The folk customs are described in more detail in this chapter, as practised by the inhabitants, and this description is accompanied by an explanation of their significance. The last chapter: *Diet,* is the most extensive and also the most detailed part of the article. In the introductory part of this chapter the author gives a detailed description of food production in relation to the religious affiliation of the population, the most frequent vegetable and animal food products raised, how the food produced was preserved and managed, and the diet of the population in relation to the season, the composition and number of daily meals, preparation of winter preserves, and preparation of the most frequent meals, as well as the opinions of the inhabitants about their diet.

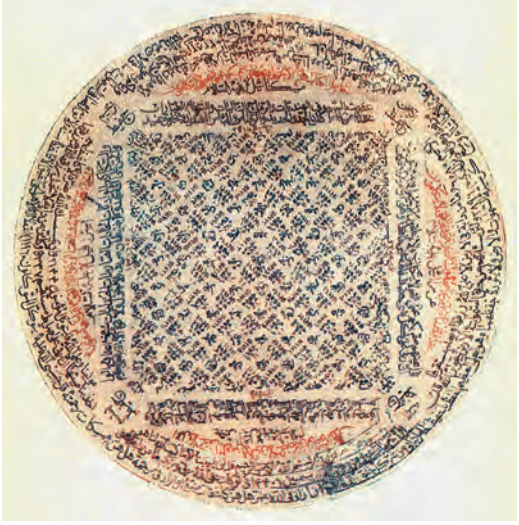
The article is illustrated with black and white sketches - grouped in eight units, under each of which there is a brief descriptive text. The group sketches are located beside the appropriate text, but are not mentioned in it. They show: various types of houses

Picture 5 Various types of houses.

(Picture 5), ground plans of Muslim, Catholic and Orthodox homes, various household objects, sketches of tattoos on the front and back of Catholic women's hands (Picture 6), graves (stone urns) with ornamental details, details of grave stones, and sketches of various amulets found in the village of Golubić. Although it is not mentioned in the article who drew these sketches, it may be assumed with great assurance that they were drawn by Dr. Stanko Sielski. There is also an original colour drawing of a apotropaic scriptures, found in the village of Golubić, allegedly from the time when the plague was rife in that region (Picture 7).



Picture 6 Tattoos of Catholic women from the village of Golubić.



Picture 7 Apotropaic scriptures from Golubić allegedly from the time when the plague was rife in that area.

Conclusion

Dr. Stanko Sielski spent his entire working life in BH, almost exclusively in the interior of the country and at a time burdened by difficult and complex social and political circumstances. This did not prevent him from devoting himself not only to his professional work, but also to scientific research and publishing. He performed his work as a physician in a humane manner, contributing to the improvement of the health of the people of BH. His publication work was based on the results of his own research work in the fields of the history of medicine, archaeology, ethnology and sociology, which all in all contributed to better knowledge and understanding, and the preservation of the material and spritiual values of the historical and cultural heritage of BH.

Conflict of interest: The author declares that he has no conflict of interest.

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Case report

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Brain abscess due to *Aggregatibacter aphrophilus* and *Bacteroides uniformis*

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Objective. The aim of this report was to describe the occurrence of a bacterial brain abscess in a healthy individual, without any predisposing condition. **Case report**. A thirteen-year old boy was admitted to the Department of Neurosurgery after the onset of vomiting, headache and dizziness. A neurological deficit was detected during the physical examination so urgent magnetic resonance imaging of the brain was performed, revealing an intrahemispheric, right positioned solitary expansive mass with ring enhancement. Purulent material was obtained during osteoplastic craniotomy with total extirpation of the brain abscess. *Aggregatibacter aphrophilus* and *Bacteroides uniformis* were isolated. The patient's general condition improved and the neurological deficit subsided as a result of the prompt recognition and treatment of this life threatening condition. **Conclusion**. To achieve a favourable clinical outcome, prompt recognition and surgical treatment of a brain abscess are of primary importance,followed by administration of appropriate antimicrobial therapy. To our best knowledge, this is the first report of this combination of microorganisms as the cause of a brain abscess.

Introduction

A bacterial brain abscess is a relatively uncommon but extremely serious and life threatening infection. The incidence ranges from 0.3-1.3/100,000 persons per year, with around 2% associated with dental infections (1, 2). Intracranial abscesses can originate from infection of contiguous structures (e.g., otitis media, dental infection, mastoiditis, sinusitis), secondary to haematogenous dissemination from a remote site (especially in patients with cyanotic congenital heart disease), after skull trauma or surgery, and, rarely, following meningitis. In at least 15% of cases, no source can be identified (3). *Aggregatibacter aphrophilus (*formerly *Haemophilus aphrophilus)* is a member of the normal flora of the human oral cavity and pharynx. It may cause brain abscess and infective endocarditis, and has been isolated from various other body sites, including the peritoneum, pleura, wounds and bone (4). *Bacteroides* spp. is generally isolated from mixed infections with other aerobic and anaerobic bacteria, forming a polymicrobial infection. Colonization of the oropharyngeal cavity can lead to the isolation of these species from brain abscesses (5).

The aim of this report was to describe for the first time the occurrence of a bacterial brain abscess due to this combination of microorganisms in a healthy child without any predisposing condition.

Case report

A healthy thirteen-old-boy was admitted to the Department of Neurosurgery at the University Hospital Centre, Osijek, due to frontally positioned headache, dizziness and vomiting. The patient was conscious, and physical examination revealed mild left hemiparesis, driftage during walking and instability in the Romberg position without signs of meningism. Terminal left diplopia and inability of terminal abduction of left bulbus were also detected during the examination of his bulbomotoric abilities. Five days before admission to the hospital, he had had a case of diarrhoea with headache, dizziness and vomiting, and was subfebrile (37.5 °C axillary measurement). He was examined by an infectious disease specialist who recommended a dietary regimen and rehydration at home. The headache did not subside and was even enhanced during movement, so urgent magnetic resonance imaging (MRI) of the brain was performed and revealed an intrahemispheric, right positioned, solitary expansive mass, diameter 41x31 mm, characteristically ring shaped with peripherally contrast enhancing, peripheral oedema and mass effect to the left (Figure 1).

Cardiovascular, respiratory and abdominal examinations revealed no abnormalities. Laboratory findings were: C-reactive protein 0.5 mg/l, erythrocyte sedimentation rate 18 mm/hour, and white blood cell count 6.6 $x10^9$/l. Only dental problems (caries) were detected. Prior dental treatment was excluded. Paediatric HIV infection is very rare in Croatia. In the period of 1985-2014 only 14 HIV positive children from HIV positive mothers were reported (6). Therefore we did not regard HIV testing to be necessary. The right parietal osteoplastic craniotomy was performed with complete evacuation of the purulent material and the abscess capsule. Empirical therapy with ceftriaxone and metronidazole was started. Samples obtained during the operation were immediately sent to a microbiology laboratory for aerobic and anaerobic cultivation. Blood agar plate and Brain-Heart infusion broth were inoculated and incubated aerobically at 37 °C/24h. Chocolate agar plate after inoculation was incubated at 37 °C in atmosphere with 5%-10% CO_2. Columbia agar and thioglycolate broth were also inoculated and incubated at 37 °C/48h in anaerobic atmosphere. Direct sample smear revealed Gram-negative bacilli with heavy polymorfonuclear infil-

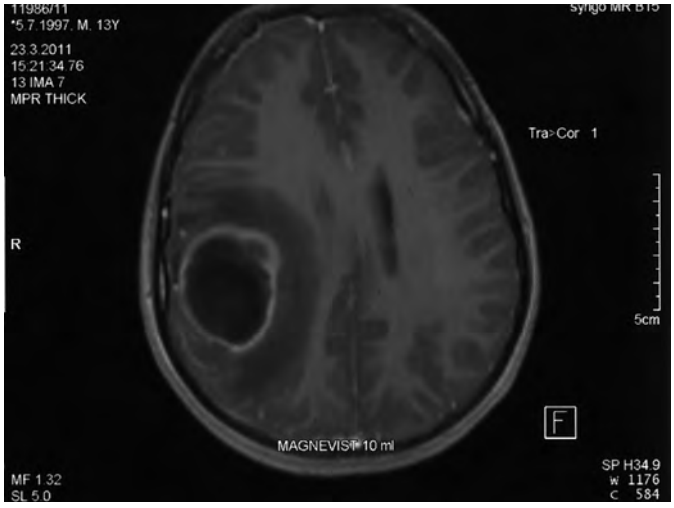

Figure 1 Characteristically ring shaped right positioned, intrahemispheric solitary expansive mass, with peripherally contrast enhancing and peripheral oedema.

tration. On the chocolate agar opaque, yellowish, catalase, urease and oxidase negative colonies of Gram-negative bacilli grew, identified by API NH system (bioMérieux, Marcy l'Etoile, France) as *Haemophilus aphrophilus*. Antimicrobial susceptibility was detected by the Kirby- Bauer disc diffusion method and the isolate was susceptible to ampicillin, amoxicillin-clavulonic acid, sulfametoxasole-thrimetoprime, ceftriaxone and meropenem, according to EUCAST breakpoint standards (7). From the material cultivated in anaerobic condition whitish colonies of non-spore forming Gram-negative bacilli were isolated. Using API 20 A system (bioMérieux, Marcy l'Etoile, France) colonies were identified as *Bacteroides uniformis*, and for antimicrobial susceptibility testing purposes ATB®ANA (bioMérieux, Marcy l'Etoile, France) was used. The strain was resistant to penicillin, clindamycin and amoxicillin, and susceptible to metronidazole, imipenem, amoxicillin-clavulonic acid, piperacillin-tazobactam and cefotaxime.

Metronidazole 4x250 mg and ceftriaxone 2x2 g were administered for sixteendays with a switch to cefixime 1x400 mg for ten days, on the recommendation of the infectious disease specialist. Control head MRI revealed substantial brain oedema regression, with minimal left ventricle compression. There was no mass effect to the left ventricle or signs of abscess residua. After the surgical drainage and antimicrobial therapy, the patient's general condition improved, with complete regression of discrete neurological deficit. Fifteen-month follow-up showed no signs of recurrence of the abscess.

Discussion

Haemophilus aphrophilus is one of the normal oral cavity flora. It is a fastidious Gram-negative bacillus part of the HACEK group, which includes *Haemophilus* species, *Aggregatibacter actinomycetemcomitans* (formerly *Actinobacillus actinomycetemcomitans), Aggregatibacter aphrophilus* (formerly *Haemophilus aphrophilus* and *Haemophilus paraphrophilus*), *Cardiobacterium hominis*, *Eikenella corrodens* and *Kingella* species involved in the cases of bacterial endocarditis with or without predisposing heart disease (8, 9). The species *Haemophilus aphrophilus* and *Haemophilus paraphrophilus* were reclassified as single species *Aggregatibacter aphrophilus*. They are Gram-negative, short regular bacilli, 0.5×1.5-1.7 μm with occasional filamentous forms. They require 5%-10% CO_2 for primary isolation. Their growth may be enhanced by haemin, but X-factor is not an absolute requirement. Some isolates require V-factor (formerly *Haemophilus paraphrophilus*), whilst others are V-factor independent (formerly *Haemophilus aphrophilus*). The colonies on chocolate agar are opaque, granular and yellowish, catalase and urease negative, oxidase variable (4). Congenital heart disease and dental procedures have been described as potential predisposing factors for infection with this organism, which can be isolated from gingival scrapings, interdental material and dental plaque (10). It is described as a cause of brain abscesses, and can develop even in otherwise healthy individuals with dental problems (11).

Bacteroides spp. is a strict anaerobe, Gram-negative bacillus, and part of the normal gastrointestinal flora. It can also colonise the oral cavity of patients with poor oral hygiene or those who have received antimicrobial therapy, especially with β-lactam agents (6). It is an important pathogen of various infections in children. The colonisation of the oropharyngeal cavity can lead to the isolation of these species from paediatric infections that originate in this area, such as aspiration pneumonia, lung abscesses, chronic otitis media, brain abscesses and subcutaneous abscesses, or burns near the oral cavity (6). *Bacteroides* spp. is generally

isolated from mixed infections with other aerobic and anaerobic bacteria, forming a polymicrobial infection (6). Organisms of the bacteroides group are often isolated from abscesses in the temporal lobe, although metastatic, haematogenous and post-traumatic abscesses can be found in different sites (12). Brain abscesses are often polymicrobial, especially if they arise from a dental source (13). Most odontogenic brain abscesses occur following dental treatment, such as periodontal therapy and the extraction of infected teeth, as the integrity of the vascular endothelium is breached. The fact that dental procedures frequently cause bacteremia and extra oral infections is well documented. However, extra oral abscesses are rare because the body's immune system expunges most odontogenic bacteremia (14).

Appropriate management of mixed aerobic and anaerobic infections requires the administration of antimicrobials that are effective against both aerobic and anaerobic components of the infection, in addition to surgical correction and drainage of the pus. The environment of an abscess is detrimental for many antimicrobials. The abscess capsule interferes with the penetration of antimicrobial agents, and the low pH and the presence of binding proteins or inactivating enzymes may impair the activity of many antimicrobials (15). From samples obtained during the intraoperative removal of the brain abscess, *Aggregatibacter aphrophilus* and *Bacteroides uniformis* were isolated. This is the first time that this combination of microorganisms was isolated from a brain abscess in our microbiology department. Reviewing the literature, we did not find any reports of the same bacteria isolated from a brain abscess.

Conclusion

Since both bacteria can be found in the normal human oral cavity, as well as the patient's good general condition, without comorbidities, we believe that in this case the brain abscess originated from an odontogenic infection. It signifies the importance of preserving good oral hygiene, otherwise a serious infection may develop even in a healthy child. In conclusion, to achieve a favourable clinical outcome, prompt recognition and surgical treatment of the brain abscess are of primary importance, followed by administration of appropriate antimicrobial therapy.

Authors' contributions: Conception and design: MB and VZA; Acquisition analysis and interpretation of data: MB, VZA and IH; Drafting the article: MB and VZA; Revising it critically for important intellectual content: DV and IH.

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Case report

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Pediatric advanced stage nasopharyngeal carcinoma - case report

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Key words: Nasopharyngeal carcinoma ▪ Rare tumors ▪ Child.

Objective. Nasopharyngeal carcinoma is an extremely rare pediatric malignancy predominantly occurring in adolescent males. Its multifactorial pathogenesis is most strongly associated with the exposure to Epstein-Barr virus in genetically susceptible hosts. In younger patients, more aggressive biological behavior has been observed, although the overall survival is better compared to adults. Due to its rarity and nonspecific clinical presentation, the diagnosis in children is often delayed and misinterpreted. **Case report**. We report a case of a 16-year-old boy with stage IVB nasopharyngeal carcinoma. He presented with a painless palpable neck mass, nasal congestion and a history of occasional epistaxis and headaches. Four years after the completion of a multimodal treatment, the patient is in complete remission. **Conclusion**. Although exceedingly rare, pediatricians should consider nasopharyngeal carcinoma in the differential diagnosis of palpable neck masses, especially in male adolescents. A multidisciplinary approach in the diagnosis, treatment, supportive care and follow-up is of utmost importance.

Introduction

Nasopharyngeal carcinoma (NPC) is a rare pediatric malignancy accounting for less than 1% of all pediatric neoplasms (1). The age-adjusted annual incidence rate increases with age, reaching its peak in adolescents (2). Its multifactorial etiology comprises genetic predisposition, epigenetic alternations related to Epstein-Barr virus (EBV) and exposure to environmental factors. Compared to adults, pediatric NPC is biologically more aggressive and generally diagnosed in advanced stages (3, 4). The current treatment protocols have resulted in excellent outcome (2). We report a diagnostic and therapeutic approach to a male adolescent with advanced stage NPC.

Case report

A 16-year-old boy was admitted to the Department of Head and Neck Surgery, Clinical Hospital Centre Rijeka, due to a one-month painless swelling on the right side of the neck. The mass showed no signs of regression following two-week antibiotic treatment. Additionally, for the previous three months the patient had been complaining of nasal congestion and obstruction, as well as occasional epistaxis and headaches, attributed to a seasonal allergy. His personal and family medical history were unremarkable.

Ultrasound showed bilateral cervical lymphadenopathy. Lymph node fine needle aspiration demonstrated carcinoma cells. The patient underwent node biopsy. His-

topathological and immunohistochemical examination set the diagnosis of undifferentiated NPC, World Health Organization (WHO) type III (Figure 1 A-D).

The boy was transferred to the Division of Hematology and Oncology, Department of Pediatrics, Rijeka, for further examination and treatment. Upon admission, he was in a good general condition, with an audible inspiratory stridor. Bilateral firm painless cervical lymph node conglomerates were palpable (left 10x6 cm, right 4x4 cm in size). Complete blood count, kidney and liver function tests, lactate dehydrogenase, ferritin and copper were within reference values, as well as the tumor markers (carcinoembryonic antigen, neuron-specific enolase, alpha-fetoprotein and beta human chorionic gonadotropin). Hypergammaglobulinemia was present with immunoglobulin (Ig) G level of 18.1 g/l (normal range 7-16 g/l). Serological testing was positive for IgG and IgA viral capsid antigen (VCA) and IgG anti-EBV nuclear antigen (EBNA); IgM anti-VCA, IgM anti-EBNA and IgG anti-early antigen (EA) were negative. No circulating plasma EBV DNA was detected. Polymerase chain reaction (PCR) identified EBV DNA in the tumor tissue.

The multislice computed tomography (MSCT) of the head and neck confirmed bilateral cervical lymphadenopathy, and revealed a nasopharyngeal tumor 37.6 x 31.7 x 25.2 mm in size (Figure 2). Detailed imaging studies (chest and spine X-ray, abdominal ultrasound, CT of the chest and the abdomen,

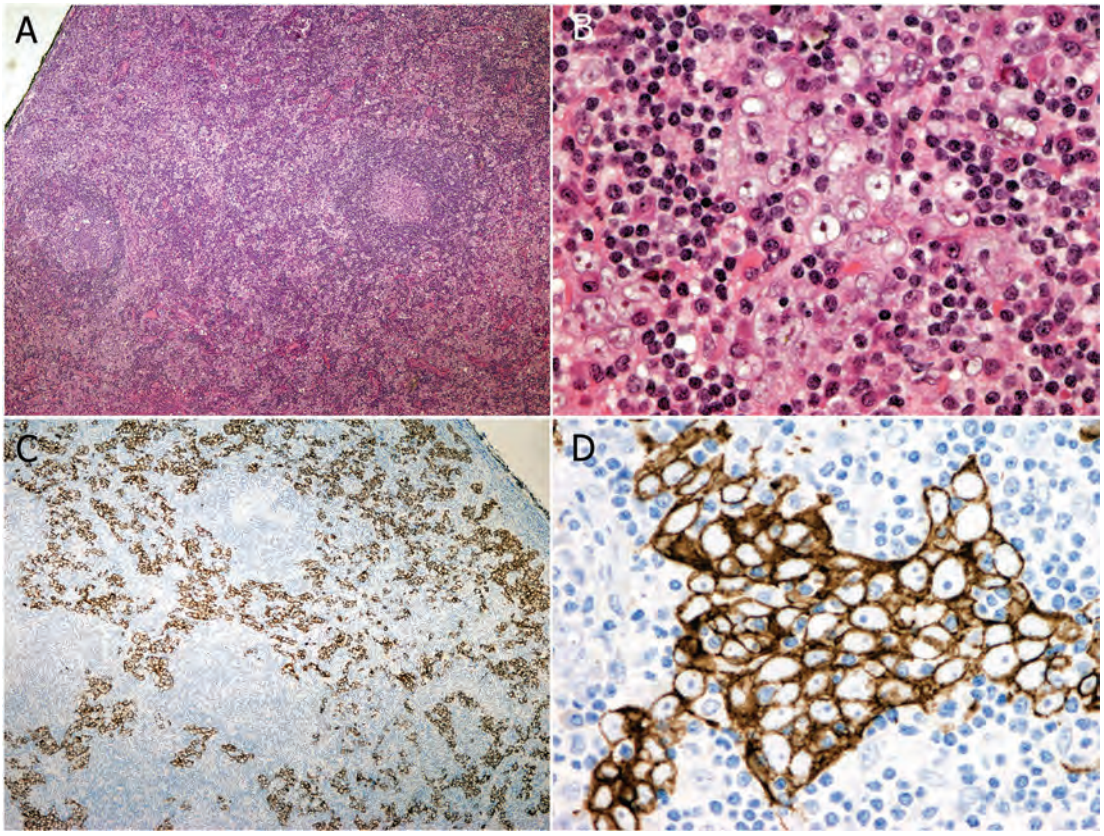

Figure 1 Metastasis of nasopharyngeal carcinoma to the lymph node. (A) Islands of tumor cells infiltrate the sinuses and lymph node parenchyma. Tumor cells exhibit large, vesicular nuclei with prominent red nucleolus and a moderate amount of pale eosinophilic cytoplasm (B, magnification x400). Immunohistochemically, tumors cells show strong cytoplasmic positivity with epithelial markers for keratin /AE1/AE3) (C, magnification x40; D, magnification x400).

bone scintigraphy) excluded the presence of distant metastases. Bone marrow aspiration was negative for malignant infiltration. A revision of the histopathological findings confirmed the diagnosis of undifferentiated NPC. According to the TNM classification, the tumor was classified as T2 (parapharyngeal extension), N3 (lymph node metastases larger than 6 cm) and M0 (no distant metastases), stratifying the patient into IVB stage and the high-risk group.

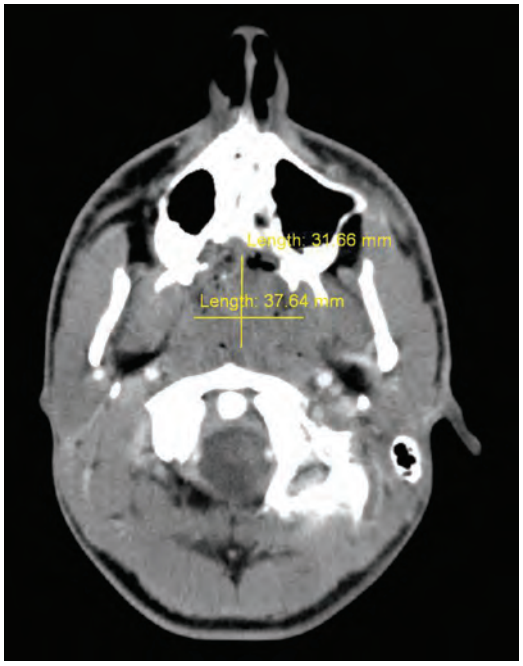

Figure 2 Multislice computed tomography (MSCT) of the head showing a nasopharyngeal tumor measuring 37.6 x 31.7 mm.

The boy was treated according to NPC-2003-GPOH protocol. He received neoadjuvant chemotherapy consisting of three courses of cisplatin (100 mg/m^2, day 1), 5-fluorouracil (1.000 mg/m^2/day, day 1-5), and folinic acid. During the treatment the patient developed deep right brachial vein thrombosis, which was treated with low molecular weight heparin for 3 months. He had several episodes of febrile neutropenia, and steroid diabetes during the neoadjuvant treatment.

Follow-up MSCT demonstrated a partial response. Photon external radiotherapy - tomotherapy was performed at the National Cancer Institute Aviano, Italy. A cumulative dose of 7000 cGy was delivered to the nasopharynx and left neck by conventional fractionation (33 fractions, 5 fractions/week), with concomitant cisplatin (20 mg/m^2, day 1-3) during the first week and the last week of irradiation. After irradiation, the patient received recombinant interferon (IFN) beta for 6 months. CT performed 6 weeks after the end of radio-chemotherapy and at the end of IFN treatment showed no evidence of a tumor. Four years after the completion of the treatment, the patient is in complete remission. He takes replacement therapy for hypothyroidism.

Discussion

NPC is an extremely rare pediatric neoplasm that arises from the epithelial cells of the nasopharynx. The age adjusted annual incidence rate increases with age, being 0.1 per million at age 0 to 9 years, 0.8 per million at age 10 to 14 years, and 1 to 2 per million at age 15 to 19 years (2). The median age at diagnosis is 15.3 years (1).

The incidence of NPC is characterized by gender, racial, ethnical and geographic variations, demonstrating its multifactorial pathogenesis. Males are two to three times more frequently affected than females (3). NPC is more common in black children (4). It is endemic in a few well-defined regions of China, Southeast Asia and Arctic region, while intermediate incidence rates are seen in North Africa and parts of the Mediterranean basin (3, 5). Studies conducted in these regions have found a positive association between consumption of salt-preserved fish containing significant levels of nitrosamines and NPC risk (3, 6). In addition, a constant link has been described between the frequency of human leukocyte antigen

(HLA) class I genes in certain populations and the risk of developing NPC (7). The strongest association has been established between NPC and EBV infection; circulating free EBV DNA is detected in more than 90% of advanced stage NPC patients. Titer levels of antibodies to EBV IgA-VCA and IgA-EA have been widely used as diagnostic and prognostic markers. Quantification of EBV DNA using real-time PCR is highly sensitive and specific for NPC, and is used in clinical management of NPC patients. High EBV DNA load at diagnosis or detectable viral load post-treatment is associated with poor survival and frequent relapse (8-10).

Pediatric NPC is different from its adult counterpart. It is biologically more aggressive and usually presents in an advanced stage. Approximately 87% of pediatric NPC is an undifferentiated subtype, which is more commonly associated with elevated EBV titer, confirming a prior viral infection in genetically susceptible children (4, 11). Furthermore, children are at higher risk of developing therapy-related complications, including secondary cancers. Nevertheless, pediatric patients have a significantly greater overall and relapse-free survival compared to adults (4, 12). The most commonly described symptoms in children are: neck mass, headache, tinnitus, hearing loss, nasal obstruction, cranial nerve palsy, diplopia and facial anesthesia. Due to the nonspecific symptoms, the median time to diagnosis is 4.8 months (13).

The treatment of pediatric NPC has undergone drastic changes in the last few decades. Until the early 1990's, patients were mostly treated by irradiation, but then chemotherapy was introduced. NPC-2003-GPOH is the current treatment protocol applied in most European countries. It includes neoadjuvant chemotherapy, radiochemotherapy and IFN beta. The results are superior to the outcomes of published results from other pediatric NPC study groups, with an event-free survival rate of 92.4% and overall survival rate of 97.1% at a median follow-up of 30 months (14). The combined therapy is well tolerated. Acute side effects are mainly leucopenia, mucositis and nausea, while chronic side effects are hearing loss and hypothyroidism (2, 15). In order to reduce toxicity, children have recently been treated by intensity-modulated radiotherapy (IMRT), which offers better volume coverage of the tumor, while protecting nearby healthy tissue (16). In addition, lower doses are administered without locoregional failures (16).

This paper presents an advanced stage, high-risk pediatric NPC. The disease initially presented as a bilateral cervical lymphadenopathy. Histopathological and immunohistochemical examination established the diagnosis of undifferentiated NPC. PCR identified EBV DNA in the tumor tissue, dictating the addition of EBV-directed immunotherapy with IFN to standard therapy. Four years after the end of the treatment the patient is in complete remission with acceptable toxicity, i.e. hypothyroidism resulting from radiotherapy. Our case indicates that NPC, although very rare, should be considered in children with solid neck masses. EBV serology as well as imaging methods are required to set up a treatment plan. Currently, the preferred imaging modality for disease staging is magnetic resonance imaging (MRI). Positron emission tomography-computed tomography (PET-CT) may underestimate the extent of the tumor and regional lymphadenopathy compared to MRI at the time of diagnosis, but is sensitive and specific for follow-up (17). Despite the low rate of relapses/recurrences, long-term follow-up is recommended, as well as endocrine monitoring, due to iatrogenic hypothyroidism.

Conclusion

NPC is a very rare pediatric malignancy characterized by its biological aggressive-

ness. The current treatment protocols have significantly improved the prognosis for young patients. Further prospective multi-institutional studies are required in order to standardize treatment approaches in children with different stages and histological subtypes of NPC, as well as to investigate the later toxicity of the treatment.

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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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Guillain-Barré Syndrome presenting as unilateral hip pain in a child

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Key words: Guillain-Barré syndrome ▪ Transient synovitis of the hip ▪ Hip pain ▪ Acute irritable hip.

Objective. The aim of this report is to highlight the importance of close observation and follow-up in children who present with an acutely irritable hip. This is because hip pain is a symptom of not only benign but also severe conditions. Thus, at the time of the initial presentation, hip pain can be misdiagnosed. This report serves as an example for a wide range of doctors such as orthopaedic surgeons, paediatricians, emergency room physicians or primary care physicians, because these are the first-line doctors who treat patients with a painful hip. **Case report.** We herein present a three-year-old child who was admitted to our hospital with pain in the right leg and initially diagnosed with transient synovitis of the hip. An additional examination two days later, after severe deterioration of the clinical picture, revealed that our patient was actually suffering from Guillain-Barré syndrome. Failure to diagnose Guillain-Barré syndrome and initiating prompt treatment is potentially life-threatening. **Conclusion.** Clinicians should be aware that hip pain could be the presenting complaint of Guillain-Barré syndrome, a syndrome that has many clinical features. Even when all the clinical and laboratory findings indicate a benign condition, Guillain-Barré syndrome should still be considered. Therefore, close observation and follow-up in children who present with an acutely irritable hip is highly recommended. In this way, the potentially catastrophic consequences of more severe conditions can be avoided.

Introduction

Guillain-Barré syndrome (GBS) is an acute, immune-mediated peripheral neuropathy. This is generally characterized by fast progressive muscle weakness and paraesthesia (1, 2). Although GBS is a relatively uncommon condition, the consequences of a missed or delayed diagnosis and delayed treatment can lead to progression of muscle weakness and a worse outcome (3). Hip pain is a symptom of both benign and severe conditions. It is suggested to be a primary complaint in paediatric GBS commonly preceding the onset of motor paralysis (4-11). The aim of this report is to highlight the importance of close observation and follow-up in children who present with an acutely irritable hip, as hip pain may be the presenting complaint in both benign and severe conditions. Thus, at the time of the initial presentation, hip pain can be misdiagnosed, resulting in potentially severe consequences.

Case report

A three-year-old girl was admitted to our hospital with pain in the right leg. The pain had started the previous day. At the time of her admission, she limped and was reluctant to walk. The pain was precipitated by walking and was vaguely localized to the right hip. In the preceding five days, the patient suffered a three-day fever that was accompanied by flu-like symptoms (e.g., coryza, headache, sore throat and productive cough). The child received symptomatic treatment at home with antipyretics. She was apyrexial for 48 hours prior to her admission. On examination, she was also apyrexial but irritable. She refused to stand or bear weight and preferred to lie down with her right hip flexed and mildly rotated externally. The child was distressed on flexion, extension and internal rotation of the right hip. The joint had a full range of movement with no swelling or tenderness. There were no signs of trauma, rashes, oedema or warmth to the limbs. A physical examination revealed normal muscle power, muscle tone and strength in the lower and upper extremities. The tendon reflexes were interpreted as normal in the extremities. Sensation was bilaterally normal. Furthermore, the cranial nerves were intact. The rest of the physical examination revealed no pathological findings. Her personal and family history revealed no specific information. Her neurological development was compatible with her age and all of her immunizations were on schedule. She had had no recent immunizations and had not been exposed to neurological agents.

Laboratory tests revealed negative inflammatory markers (CRP and leukocyte count). The X-ray of the right hip joint showed no pathological findings. The ultrasound of the right hip revealed a small amount of effusion without synovial thickening. The more severe causes of hip symptoms, such as septic arthritis, osteomyelitis, Legg-Calve-Perthes disease, juvenile idiopathic arthritis, fractures and tumours, were excluded from the diagnosis with imaging and laboratory investigations. Normal neurological examination excluded neurological conditions such as GBS. The most likely diagnosis was transient synovitis of the hip. The treatment consisted of rest and anti-inflammatory agents. A follow-up examination was arranged for three days later.

However, two days after the first admission, the child presented a clinical deterioration with ataxic walking and inability to walk independently. This resulted in her readmission to our hospital. On examination, lower limb weakness and hypotonia were found, as well as minimal antigravity movements in the lower limbs. A neurological examination revealed isochoric pupils, papillary light reflexes bilaterally positive, muscle strength 5/5 at neck flexor muscles, 5/5 at distal and proximal muscles of the upper extremities and 3/5 at distal and proximal muscles of the lower extremities. There were no clonuses or tremors. An examination of the upper limbs revealed normal tone and power. Reflexes of the biceps, triceps and supinator were interpreted as normal. On the other hand, the tendon reflexes were bilaterally reduced in the lower extremities. The patella and ankle reflexes were diminished bilaterally. Plantar reflex response was flexion. The intestine and bladder sphincters were intact. Autonomic functions were normal. There was no loss of touch or pins-and-needles sensation. There was coughing. Examinations of the lungs and rest systems were normal. Biochemical laboratory tests with ammonia and lactate, as well as haematological tests, showed no pathological findings. A retinal examination revealed no specific findings. In the MRI of the brain, no pathology was observed at the brain stem. Furthermore, the whole spine MRI after a gadolinium injection showed no pathological findings. There were no microbial proliferations in cultures of blood, urine, stool

Table 1 Nerve conduction studies

Type of nerve	Name of nerve	Latency	CMAPs	NCV
		R/L, ms; (Range)	R/L; ms (Range)	R/L; ms (Range)
Motor	Median	2.5/2.6; (1.7-3)	4.8/4.9; (4-12)	49.5/49.6 (49-73)
	Ulnar	2.1/2; (1.3-2.4)	5.2/5.4; (5-18)	49.7/49.9; (49-65)
	Peroneal	2.4/2.5; (2.2-4)	1.2/1.1; (2-12)	41.2/41.4; (40-60)
	Tibial	2.5/2.6; (2-4)	1/1.1; (2-12)	43.8/43.9; (43-57)
Sensory	Median	2/2.1; (1.8-2.5)	38.4/38.6; (17-85)	55.2/56.3; (53-75)
	Ulnar	1.9/2.1; (1.7-2.4)	53.8/53.9; (15-62)	56/56.7; (53-79)
	Sural	1.9 /1.8; (1.7-2.4)	26.0 /47.6; (8-47)	44.5/45; (44-59)

R/L= Right/Left; CMAPs=Compound muscle action potentials; NCV=Nerve conduction velocity.

and cerebrospinal fluid. There were no leukocytes in the cerebrospinal fluid and protein as well as glucose were in the laboratory reference range. The results of the serum immunoglobulins were normal. The neostigmine test was negative. The serologic tests were negative for the herpes virus, Epstein-Barr virus, cytomegalovirus, rubella, rubeola, toxoplasmosis, enteroviruses, respiratory viruses, Lyme disease and *Mycoplasma pneumoniae*. There were no microbial proliferations in the culture for *Salmonella*, *Shigella* and *Campylobacter jejuni*. The child finally underwent nerve conduction studies (NCSs). The findings of motor NCSs in the lower limbs showed markedly reduced amplitude of compound muscle action potentials (CMAPs), whereas distal latencies and motor conduction velocities were normal. The findings in the upper limbs were interpreted as normal, as were the findings of sensory NCSs (Table 1). Unfortunately, anti-ganglioside antibodies, which could be informative, were not possible to be tested in our laboratory.

Based on clinical features with motor involvement, electrophysiological investigation, as well as the normal laboratory findings, this patient was diagnosed with acute motor axonal neuropathy (AMAN) subtype of GBS or paraparetic GBS according to the Wakerley et al. classification (9). Lee et al. reported the first case of AMAN confirmed by electrophysiological studies that was accompanied by severe pain of the entire body (8). They suggested that clinician should be on the alert to atypical sensory symptoms from the classical presentation of AMAN even if the patient is diagnosed with AMAN electrophysiologically and should consider proper treatment options based on clinical presentations (8). Our diagnosis was strengthened according to this report and the patient was treated with 0.4 g/kg/day of intravenous immunoglobulin for five days. She did not require respiratory support and recovered slowly. Three months later, at the outpatients' follow-up, she had fully recovered.

Discussion

As hip pain is the presenting complaint in many conditions, it is important that it is recognized and diagnosed in childhood. The initial presentation of an acutely irritable hip in a child can pose a diagnostic challenge to an orthopaedic surgeon, paediatrician, emergency-room physician or primary-care physician. The differential diagnosis includes both benign and severe conditions such as transient synovitis of the hip, Legg-Calve-Perthes disease, osteomyelitis, septic arthritis, trauma and neurological conditions such as GBS (3). At the presentation time, our patient's clinical examination,

imaging tests and blood tests excluded the more serious clinical conditions and the diagnosis of transient synovitis of the hip was established. As a result, our patient was treated in an outpatient setting with oral analgesics and rest.

Transient synovitis of the hip is the most common cause of hip pain in children. Since transient synovitis has been associated with the risk of subsequent Perthes disease, clinical and a possible radiological or laboratory review were recommended to our patient three days after the first admission (12, 13). However, our patient's clinical condition deteriorated two days later. Our neurological examination at that time and the normal laboratory tests contributed to our differential diagnosis of GBS. GBS and more specifically the AMAN subtype was finally established, taking into account our neurological examination, the normal laboratory and imaging investigation and the findings of the NCSs. It was suggested that in approximately 40% of patients, NCSs performed within the first week can suggest a diagnosis of neuropathy without fulfilling the criteria for one of the specific electrophysiological subtypes (9, 14). Furthermore, although albuminocytologic dissociation in cerebrospinal fluid is a typical finding in GBS, elevations of protein usually occur in the second or third week of illness in AMAN and, as with our patient, patients with AMAN may have normal cerebrospinal fluid protein in the first week (8, 15). Hence, the appropriate treatment for GBS was started.

GBS is considered to be an acute immune-mediated neuropathy with several variations: a classic demyelinating form, acute inflammatory demyelization polyneuropathy (AIDP), acute motor–sensory axonal neuropathy (AMSAN), acute motor axonal neuropathy (AMAN) and Miller–Fisher syndrome (8, 16). Wakerley et al. from the GBS Classification Group presented clinical criteria to enable neurologists and non-neurologists to diagnose GBS and all its variants using a simple yet all-inclusive classification system (9). GBS is characterized by autoimmune attack on the peripheral myelin proteins of nerve roots and peripheral nerves. It is a relatively uncommon condition. Nevertheless, it is the most common cause of acute general paralysis in developed countries (3). In AMAN subtype, the pathological features differ from the features of AIDP in that macrophages invade the space between the Schwann cell and axon, leaving the myelin sheath intact (17, 18). Griffin et al. proposed the attractive hypothesis that AMAN and AMSAN are part of the spectrum of a single type of immune attack on the axon (18, 19). Among several variations, AMAN is characterized clinically by nearly pure motor syndrome without sensory involvement and final diagnosis of AMAN, as in our patient, is based on electrophysiological findings such as decreased CMAPs without any evidence of demyelination or change in sensory nerve action potential (SNAP). Despite the fact that AMAN has not been reported on atypical symptoms, Lee et al. described the first case of AMAN with sensory symptoms. More specifically, a 3-year-old male who was admitted with bilateral leg weakness and severe lower back and leg pain was finally diagnosed with AMAN (8). AMAN is reported more commonly in China than western countries and the majority of northern Chinese patients with GBS were classified as having AMAN (8, 20). Although most children with GBS usually have a benign and relatively limited clinical illness, failure to diagnose GBS and to initiate prompt treatment with plasmapheresis or intravenous immunoglobulin could lead to progression of muscle weakness and a worse outcome.

It is suggested that pain constitutes a common and often severe symptom in the whole spectrum of GBS (including MFS, mildly affected, and pure motor patients (11). Similar to our case, it has been also

reported as an atypical sensory symptom of AMAN subtype of GBS (8). Furthermore it has been suggested that, as it frequently occurs as the first symptom, before the onset of motor paralysis, pain in GBS requires full attention (11). It is likely that sensory nerve fibre involvement results in more severe pain (11). However, in daily clinical practice it is still considered to be an uncommon presenting symptom of GBS. This can lead to misdiagnosis or delayed diagnosis of this variant of GBS. Similarly to our case, many children who presented with pain as an early symptom of GBS were initially diagnosed with a musculoskeletal disease (4). Mahmoud et al. investigated the role of clinical presentation scaling in GBS to predict patients' short-term outcome. They found that all children experienced some degree of pain, which was mild to severe (5). Nguyen et al. retrospectively reported on a series of children under the age of six years with GBS (6). They found that pain was a symptom in all of the children at some time during their hospital stay. In their study, on admission, pain was present in 23 out of 29 patients (79%). Pain was often the most important symptom and led to misdiagnosis in 20 patients (69%). Linden et al. analysed the epidemiologic, clinical, laboratory and development profile of GBS series studied at a Child Institute, between 1989 and 2000. They found that pain was present in 62.3% (38/61) of the patients, more frequently occurring in the inferior members. They stated that it was an important cause of irritability in the smaller children. In some cases, this led to a delay in diagnosis (7).

On the other hand, despite the fact that we considered the pain as the presenting symptom of GBS that preceded the onset of motor paralysis, it cannot be denied that the hip pain in our patient may have occurred because of the preceding viral infection. However, according to strong evidences in the literature, pain could be the presenting complaint of GBS. Additionally, as previously stated, Lee et al. described the first case report of AMAN confirmed by electrophysiological studies that was accompanied by severe pain of the entire body. They concluded that clinician should be on the alert to atypical sensory symptoms from the classical presentation of AMAN even if the patient is diagnosed with AMAN electrophysiologically (8).

Furthermore, the AMSAN subtype of GBS was excluded because of a number of reasons. Firstly, in our case the findings of sensory NCSs were without pathological findings and except from the presenting symptom of pain there were no other sensory symptoms or sensory clinical findings which could indicated AMSAN subtype of GBS. Additionally, similar to our case, AMAN is characterized by rapidly progressive weakness, and usually good and full recovery, (our patient three months after the presentation, at the outpatients' follow-up, she had fully recovered) while AMSAN is generally associated with slow and incomplete recovery (21, 22). Because of these reasons as also the fact that the number of GBS-subtype AMSAN cases is very small (< 10% of AMAN cases) and in children even smaller the most likely diagnosis in our case was AMAN subtype (23). Therefore, we considered that our patient was presented with atypical sensory symptoms (hip pain) and we strongly believed that hip pain was the presenting symptom of the AMAN subtype of GBS.

Therefore, our case demonstrates that children with painful hips should be under observation. Taking into account that the common symptom could also be part of an uncommon syndrome, a follow-up a few days later should always be recommended. It is important that all clinicians recognize the value of both serial physical examinations and ongoing critical interpretations of the acquired clinical and laboratory data.

In this way, they can adjust a tentative diagnosis towards a potentially life-threatening disease.

Conclusion

Pain of the lower limb can be a predominant symptom in both benign and severe conditions. Thus, misdiagnosis at the time of initial presentation can occur. Clinicians should be aware that hip pain could be the presenting complaint of GBS, a syndrome that has many clinical features. Even when all the clinical and laboratory findings indicate a benign condition, GBS should be considered. Therefore, close observation and follow-up in children who present with an acutely irritable hip are highly recommended. In this way, the potentially catastrophic consequences can be avoided.

Authors' contributions: Conception and design: CN, KD and KM; Acquisition analysis and interpretation of data: CN, KD, EV and KT; Drafting the article: CN and AA; Revising it critically for important intellectual content: CN and AA.

Consent: Written informed consent was obtained from the parents of the patient for publication of this case report.

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Commentary

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Obtaining a PhD: Personal experience of a nurse

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Key words: Nursing ▪ Education ▪ Encouragement and cooperation ▪ Mentoring ▪ Research.

I read with great interest the article by Sambunjak (1) on mentoring, and the reactions it evoked. It prompted me to offer my own experience as a nurse attending a postgraduate program, with hopes that it may serve the worldwide community of nurses through encouragement, advice and vivid description.

First, I want to tell my colleagues not to be afraid of postgraduate studies. Nursing needs to rise to higher levels than it is today, as it plays an important role in ensuring safe and effective patient care. Drawing upon evidence based medicine, nursing needs to carry out competitive and high quality research. And in order to do so, nurses need to engage and adopt scientific and critical thinking, and partake in cooperation and exchange of experiences. In Croatia, after finishing nursing school, which is a secondary school that students usually enter at age 13-14, nursing education can be continued on three levels: pregraduate (baccaleurate), graduate (master's level), and postgraduate (doctoral) (2).

I did my master's thesis on the attitudes towards sexual health and contraception and my mentor was prof. A. M. Several months later, her husband prof. M. M. asked me: "What's next?" and advised I continue my education at the postgraduate program, where, if I was interested, I would be able to deepen my knowledge and acquire new skills by engaging in research, and learning truly what it means to develop a topic, do literature search, apply constructive criticism, and collect and analyse data. I was unsure at the time, and when he asked me: "Where do you see yourself in the future – what would you like your lifelong work to be? I replied: "Working with mothers and children." Immediately he said: "Great, let's go to the Department of Family Medicine, where a very capable researcher has finished a PhD on the subject." I didn't really even have time to think, or acknowledge my fear, when there she was, at her desk, doctor IZG. We were introduced, and professor asked her out of the blue if she was willing to be my mentor. She, as was I, was caught by surprise, and before she could give an answer, the professor's wife comes in, he tells her the plan, and she says: "Dear I., Drita was my student, I mentored her master thesis, and I can say with certanty you will not regret if you accept mentoring". And so I was given my first task, by my new mentor(s), to write a research

plan of the format that medical schools students write at the School of Medicine in Split for their theses (3). Walking to the car, I felt tears running down my face. I was terrified, in my head swarmed all kinds of thoughts – and none of them were reassuring.

But, after a few meetings, a title emerged: "The effect of educational interventions on the practice of breastfeeding in primipara: a prospective, randomized controlled trial." In drafting the protocol, I had help from a young colleague M. Mal and I learned to follow the CONSORT guidelines (3), to describe in detail the intervention, and check for the possibility of systematic errors, or biases that could effects the results (4). Then, it was time to carry out the literature search on the subject. With the help of A. U., head of the Central Medical Library at the School of Medicine, I had in front of me 496 abstracts that had to be read (at the time I was shocked, but later I even had to go through some 2000). My mentor advised me to summarize all these articles in a table, and list several characteristics of each, including the intervention, its effects, and the limitations of the study. And so I read, and learned.

In December 2011 came the call for admission to Translational Research in Biomedicine programme. By then I had already developed my research topic and almost the entire study protocol. But I was scared when I was scheduled for the interview for admission. What was I to say? I stopped by at professor's M.M. office for encouragement, but what I got was strictness and an objection to the fact that my proposal had two typographical errors: "The future scientist should not have mistakes in the text." I felt tears gathering and asked: "Will the interview be very difficult?" He waved his hand and said: "You do not have to be afraid, just tell them why you want it and what is your plan". Then he noticed my tears, and consoled me in his typical manner: "Why do you wear makeup if you are going to cry?" The interview, needles to say, went really smoothly. In February I was accepted. And then the studying, socializing, and tears began anew. Each year, we had tests, and two reports of our progress which we needed to present to the heads of the program and our colleagues.

Still there was a lot I did not know; my mentor and I were not sure whether our study should be single or double blind, how to develop a leaflet about the benefits of breastfeeding and how to approach the mothers about the study. And so we asked for help, from three different individuals, all knowledgeable about one particular thing. A month went by, and professor P. H. came one day from England to give a lecture on qualitative research. She had conducted a series of studies on breastfeeding and advised us to record all the conversations I would have with the mothers, and also told us to register the study. As our plan was now finished, we obtained the positive opinion from the Ethics Committee of the Medical School and in July 2014, we entered our study in the registry ClinicalTrials.gov. Just this month, we finished writing the full protocol in English, and have sent it out for publication.

After registration of the study, I arranged with gynaecologists in nine clinics to enable me to recruit pregnant women. At first, it worked well, but as time went on some gynaecologists, pressured with their work, irregularly sent data I needed. So I started to visit them regularly and call them up, and I am still doing it. It is hard, and takes a lot of time, but it gets the work done. Once I obtain the demographic data, I randomize women according to a predefined protocol; open a file for each one, with their name, intervention group, gynaecologist, address, phone number, and date of interventions and calls that I need to make. And even though the study is big and complicated, and I am doing repeated measurements three times, listening to mothers while they talk about their children and their desire to provide the best for them

is inspiring. I have included 300 women in the study, did more than 300 telephone interventions, and received some 360 text messages from mothers asking me advice.

In my second year professor M. M. asked: "Is it hard?". But I needed a second to reply, to think, cause I did not even have the time to think about that. "Yes, it is difficult" I said, "but it can be done." Through the hardship, you meet so many kind, helpful, and wonderful people, you get to work with respected professors and colleagues, and see the same fright and tears in new generations that I used to have. And that is perhaps the biggest treasure of it all, a new world of experiences opens. And that is my second advice, if we want nursing to evolve, we need to evolve with it.

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An unusual communication between the trunk of the mandibular nerve and the lingual nerve in a female cadaver

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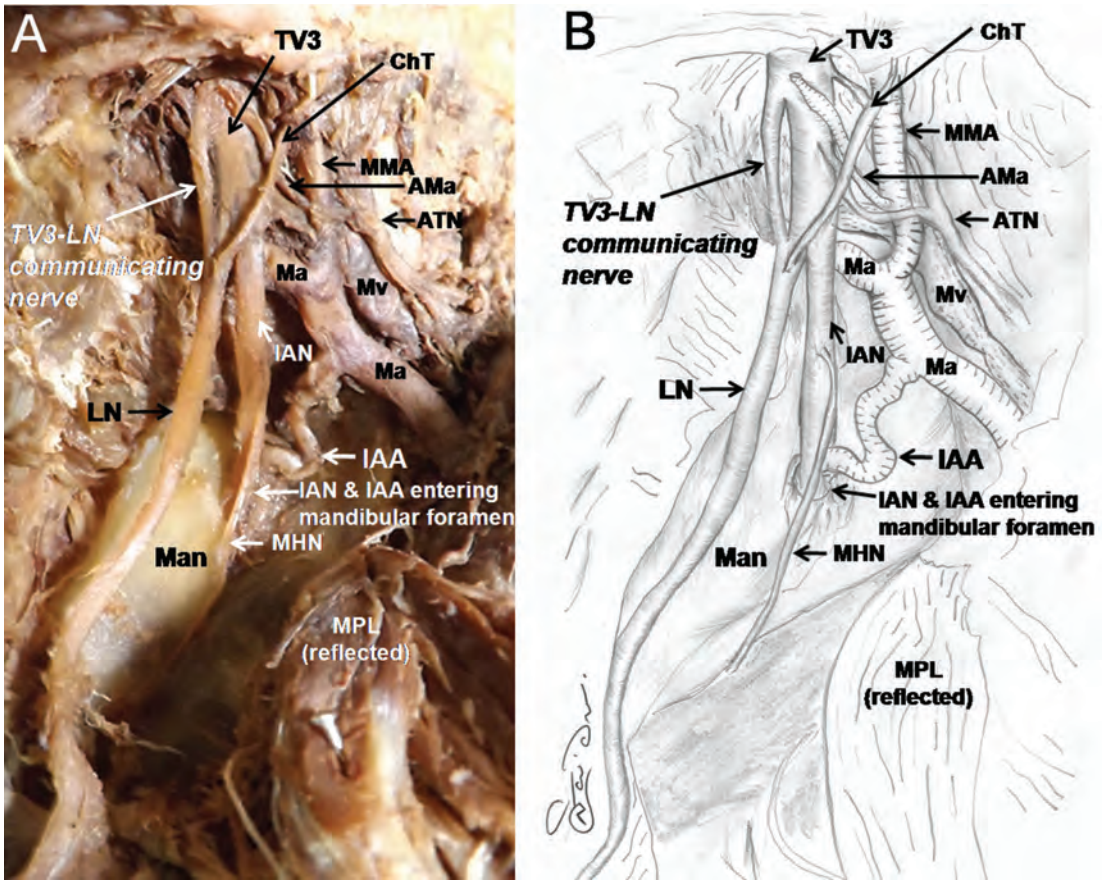

Figure 1 A photograph (A) and an illustration (B) showing the left-medial aspect of the unusual communication between trunk of mandibular nerve (TV3) and the lingual nerve (LN) communication in a 71 year old woman. ChT: chorda tympani nerve; MMA: middle meningeal artery; AMa: accessory meningeal artery; ATN: auriculo-temporal nerve; Ma: maxillary artery; Mv: maxillary vein; IAA: inferior alveolar artery; IAN: inferior alveolar nerve; MHN: mylohyoid nerve; MPT: medial pterygoid muscle; Man: mandible.

In general, TV3 emits 6 branches from the anterior and posterior division of its trunk. Branches of these divisions supply the meninges, the tensor tympani and tensor veli palatini muscles, the muscles of mastication, the anterior belly of digastric and mylohyoid muscles, the salivary glands, teeth and gingivae, and the maxillary sinus including the sensory innervation of the face. Anomalous communications among the branches of the TV3 have been clinically documented in Koreans (1), Turks (2), and Indians (3) because they can possibly explain the unsuccessful anesthesia before surgery in the infratemporal and mandibular region or the oral cavity. Common variations reported are communications of the LN with the MHN or the ATN (1-3). It was also mentioned that the IAN could communicate with the ATN or the LPN (1). Exceptionally, Erdogmus et al. (2) demonstrated an unusual communication between the LN and the MPN. Variant communications (such as the LN to the IAN or the ATN) as previously documented in several populations, may also be observed in Thai cadavers during the gross anatomy dissection. Specifically, from 102 samples (51 heads), we found a very rare and unusual communication between the TV3 and the LN (TV3-LN communication) in a 71 year old Thai woman. This communication is reported for the first time. It seems to be a posterior division of the TV3 that communicates above the junction between the LN and the chorda tympani nerve (Figure 1). This uniquely anomalous communication should be noted and documented in cases of anesthesia in the mandibular and infratemporal region, or the oral cavity in various treatment procedures.

Key words: Mandibular nerve trunk ▪ Lingual nerve ▪ Nerve communication.

Authors' contributions: Conception and design: SI; Acquisition, analysis and interpretation of data: SI, JS, PB; Drafting the article: SI; Revising it critically for important intellectual content: SI, PB.

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by Nerma Tanović

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Abstract and Key words are written on the second page. Because abstracts are the only substantive portion of the article indexed in many electronic databases, and the only portion many readers read, authors need to be careful that abstracts reflect the content of the article accurately. An abstract (250 words) is written without authors' names and institutional affiliations. Its structure should be similar to that of the text. For original articles, the abstract needs to have the structure with the following subtitles: Objective, Materials and methods, Results and Conclusion. Abstracts for Case reports also need to have the following subtitles: Objective, Case report, and Conclusion and for Review articles: Objective, Background, Methods, Discussion and Conclusion. Abstracts for Short communication (150 words) should not be structured but should end with Conclusion. Following the abstract, authors provide, and identify as such, 3 to 5 key words or short phrases that capture the main topics of the article. The key words should not repeat the title of the manuscript. Terms from the Medical Subject Headings (MeSH) list of Index Medicus should be used; MeSH terms are available from: www.nlm.nih.gov/mesh/.

Third page

Should carry the manuscript of article. Text should be under the following headings:

Introduction. Needs to be short and to specify to the reader, clearly and with arguments, reasons for the research presentation, and the novelties that the article brings. In Introduction maximum 3 to 4 pertinent and directly related works need to be cited. At the end of Introduction, an author needs to clearly specify the set aim of the research.

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Authors' contributions (eg.): Conception and design: MK and OG; Acquisition, analysis and interpretation of data: MK and GL; Drafting the article MK; Revising it critically for important intellectual content: GL and OG).

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Sample references

Articles in journals

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> Halpern SD, Ubel PA, Caplan AL. Solid-organ transplantation in HIV-infected patients. N Engl J Med. 2002;347(4):284-7.

More than six authors:

> Rose ME, Huerbin MB, Melick J, Marion DW, Palmer AM, Schiding JK, et al. Regulation of interstitial excitatory amino acid concentrations after cortical contusion injury. Brain Res. 2002;935(1-2):40-6.

Organization as author:

> Diabetes Prevention Program Research Group. Hypertension, insulin, and proinsulin in participants with impaired glucose tolerance. Hypertension. 2002;40(5):679-86.

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21st century heart solution may have a sting in the tail. BMJ. 2002;325(7357):184.

Volume with supplement:

Geraud G, Spierings EL, Keywood C. Tolerability and safety of frovatriptan with short- and long-term use for treatment of migraine and in comparison with sumatriptan. Headache. 2002;42(Suppl 2):S93-9.

Issue with supplement:

Glauser TA. Integrating clinical trial data into clinical practice. Neurology. 2002;58(12 Suppl 7):S6-12.

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Banit DM, Kaufer H, Hartford JM. Intraoperative frozen section analysis in revision total joint arthroplasty. Clin Orthop. 2002;(401):230-8.

Letters or abstracts:

Tor M, Turker H. International approaches to the prescription of long-term oxygen therapy [letter]. Eur Respir J. 2002;20(1):242.;
Lofwall MR, Strain EC, Brooner RK, Kindbom KA, Bigelow GE. Characteristics of older methadone maintenance (MM) patients [abstract]. Drug Alcohol Depend. 2002;66 Suppl 1:S105.

Article republished with corrections:

Mansharamani M, Chilton BS. The reproductive importance of P-type ATPases. Mol Cell Endocrinol. 2002;188(1-2):22-5. Corrected and republished from: Mol Cell Endocrinol. 2001;183(1-2):123-6.

Article with published erratum:

Malinowski JM, Bolesta S. Rosiglitazone in the treatment of type 2 diabetes mellitus: a critical review. Clin Ther. 2000;22(10):1151-68; discussion 1149-50. Erratum in: Clin Ther. 2001;23(2):309.

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Yu WM, Hawley TS, Hawley RG, Qu CK. Immortalization of yolk sacderived precursor cells. Blood. 2002 Nov 15;100(10):3828-31. Epub 2002 Jul 5.

Books and other monographs

Personal author(s):

Murray PR, Rosenthal KS, Kobayashi GS, Pfaller MA. Medical microbiology. 4th ed. St. Louis: Mosby; 2002.

Editor(s), compiler(s) as author:

Gilstrap LC 3rd, Cunningham FG, VanDorsten JP, editors. Operative obstetrics. 2nd ed. New York: McGraw-Hill; 2002.

Organization(s) as author:

Royal Adelaide Hospital; University of Adelaide, Department of Clinical Nursing. Compendium of nursing research and practice development, 1999-2000. Adelaide (Australia): Adelaide University; 2001.

Chapter in a book:

Meltzer PS, Kallioniemi A, Trent JM. Chromosome alterations in human solid tumors. In: Vogelstein B, Kinzler KW, editors. The genetic basis of human cancer. New York: McGraw-Hill; 2002. p. 93-113.

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Christensen S, Oppacher F. An analysis of Koza's computational effort statistic for genetic programming. In: Foster JA, Lutton E, Miller J, Ryan C, Tettamanzi AG, editors. Genetic programming. EuroGP 2002: Proceedings of the 5th European Conference on Genetic Programming; 2002 Apr 3-5; Kinsdale, Ireland. Berlin: Springer; 2002. p. 182-91.

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Borkowski MM. Infant sleep and feeding: a telephone survey of Hispanic Americans [dissertation]. Mount Pleasant (MI): Central Michigan University; 2002.

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Tynan T. Medical improvements lower homicide rate: study sees drop in assault rate. The Washington Post. 2002 Aug 12;Sect. A:2 (col. 4).

Dictionary and similar references:

Dorland's illustrated medical dictionary. 29th ed. Philadelphia: W.B. Saunders; 2000. Filamin; p. 675.

Electronic material

CD-ROM:

Anderson SC, Poulsen KB. Anderson's electronic atlas of hematology [CD-ROM]. Philadelphia: Lippincott Williams & Wilkins; 2002.

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Chason KW, Sallustio S. Hospital preparedness for bioterrorism [videocassette]. Secaucus (NJ): Network for Continuing Medical Education; 2002.

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Abood S. Quality improvement initiative in nursing homes: the ANA acts in an advisory role. Am J Nurs [serial on the Internet]. 2002 Jun [cited 2002 Aug 12];102(6):[about 3 p.]. Available from: http://www.nursingworld.org/AJN/2002/june/Wawatch.htm.

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Homepage/Web site:

Cancer-Pain.org [homepage on the Internet]. New York: Association of Cancer Online Resources, Inc.; c2000-01 [updated 2002 May 16; cited 2002 Jul 9]. Available from: http://www.cancer-pain.org/.

Part of a homepage/Web site:

American Medical Association [homepage on the Internet]. Chicago: The Association; c1995-2002 [updated 2001 Aug 23; cited 2002 Aug 12]. AMA Office of Group Practice Liaison; [about 2 screens]. Available from: http://www.ama-assn.org/ama/pub/category/1736.html.

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Who's Certified [database on the Internet]. Evanston (IL): The American Board of Medical Specialists. c2000 – [cited 2001 Mar 8]. Available from: http://www.abms.org/newsearch.as.

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1. Freckmann G, et al, System Accuracy Evaluation of 43 Blood Glucose Monitoring systems for Self-Monitoring of Blood Glucose According to DIN EN ISO 15197, J Diabetes Sci Technol, 2012; 6(5):1060–75.
2. International Organization for Standardization. In vitro diagnostic test systems – requirements for blood-glucose monitoring systems for self-testing in managing diabetes mellitus. ISO 15197:2013.

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