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Changes in lipid peroxidation, glutathione, ascorbic acid, vitamin E and antioxidant enzymes in patients with ovarian cancer

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Objective. This work was undertaken to assess oxidative stress and antioxidant status in patients with ovarian cancer. **Patients and Methods.** The study was conducted in thirty-eight patients with ovarian cancer, the control group being 38 healthy volunteers. Erythrocyte lipid peroxidation products (MDA), glutathione (GSH), ascorbic acid, plasma vitamin E and activities of antioxidant enzymes super oxide dismutase (SOD), glutathione peroxidase ($GP_{X)}$, catalase in erythrocytes and plasma glutathione – S – transferase (GST) were estimated in ovarian cancer patients. **Results.** In this study it was observed that there was a significant increase in erythrocyte MDA levels, SOD, GP_X and plasma GST activities and a significant decrease in erythrocyte GSH, ascorbic acid, plasma vitamin E levels and catalase activity in patients with ovarian cancer when compared to controls. **Conclusions.** The results of our study suggest higher oxygen free radical production, evidenced by increased MDA and decreased GSH, ascorbic acid, vitamin E and Catalase activity, as support to the oxidative stress in ovarian cancer. The increased activities of antioxidant enzymes may be a compensatory regulation in response to increased oxidative stress.

Key Words: Ovarian cancer, Malondialdehyde, Glutathione, Superoxide dismutase, Catalase, Glutathione peroxidase, Glutathione -S - transferase.

Introduction

Oxidative stress is the imbalance between the generation of reactive oxygen species and the activity of antioxidant defenses. Severe oxidative stress causes cell damage and cell death and it has been implicated in numerous human diseases including cancer (1). Lipid peroxidation mediated by free radicals is considered to be the major mechanism of cell membrane destruction and cell damage. Free radicals are formed in both physiological and pathological conditions in mammalian tissues (2). The uncontrolled production of free radicals is considered to be an important factor in the tissue damage induced by several pathophysiological processes (3). Moreover the body's defense

mechanisms would play a role in the form of antioxidants and try to minimize the damage, adapting themselves to the stressful situation. Antioxidants are compounds that dispose, scavenge, and suppress the formation of free radicals, or oppose their actions (4) and two main categories of antioxidants are those whose role is to prevent the generation of free radicals and those that intercept any free radicals that are generated (5). They exist in both the aqueous and membrane compartment of cells and can be enzymes or non-enzymes. Alteration in the oxidant -antioxidant reaction, relationship profile is known to occur in cancer (6).

Ovarian cancer remains one of the most lethal of all gynaecologic malignancies, accounting for more deaths than cervical and uterine cancers (7). More than 60% of patients with ovarian cancer do not present until they are at an advanced stage and the average 5 year survival rate is reported to be lower than 20% (8).

Patients and Methods

Blood samples were obtained from thirty-eight patients (mean age: 56 ± 10 years) with clinically and histopathologically proven ovarian cancer. Thirty-eight normal healthy age matched women volunteers served as controls. Written consents were also obtained from the patients prior to the study and the objectives of the study were fully explained. Ten of the participants were excluded from the study because they were not comfortable with the research protocol. An equal number of age matched healthy subjects were also investigated.

The complete clinical and personal history of the subjects was recorded. The subjects ranged in age between 46 – 65 years. All the patients in the study were clinically diagnosed as patients with ovarian cancer. None of these subjects were alcoholics or chronic smokers and did not suffer from any systematic diseases like hypertension or any diabetic complication. Subjects who had no other cancers and subjects with normal nutritional habits without supplementing any vitamins over 6 months were included. Subjects with a history of receiving anti-inflammatory drugs in the last 6 months and a history or present symptoms of any other stress induced disorder were excluded.

The controls and patients were divided into two groups.

- Group 1 (Controls): Thirty-eight healthy age matched women as controls.
- Group 2 (Study Subjects): Thirty-eight patients with clinically and histopathologically proven ovarian cancer.

Heparinised venous blood samples from these subjects "before meal intake" were used for the analysis. Plasma was separated by centrifugation at 1,000 g for 15 minutes. Separated plasma was used for the estimation of vitamin E and measurement of GST activity. The buffy coat was removed and the packed cells were washed three times with physiological saline. The erythrocyte suspension was prepared by the method of Dodge et al., (9) modified by Quist (10). The packed cells were used for the analysis of GSH, ascorbic acid, MDA, SOD, Catalse, GP_X. Erythrocyte GSH was estimated by the method of Beutler et al (11) using Di Thio Bis Nitro Benzoic acid (DTNB). Ascorbic acid levels were estimated by the method of Tietz (12). Plasma vitamin E levels were estimated by the method of Baker H et al (13). MDA was determined by the measure of TBARS (14). SOD (EC 1.15.1.1) activity was determined in the hemolysate by the method of Misra & Fridovich based on the inhibition of auto oxidation of epinephrine to adenochrome at Ph 10.2 (15). Catalase (EC 1.11.1.6) activity was measured by the method of Beers and Sizer (16). The activity of Glutathione Peroxidase (GPX, EC 1.11.1.9) was measured as described by Paglia and Valentine (17) in erythrocytes and

activity of GST (EC 2.5.1.18) was measured by using 1-Chloro-2, 4-Dinitro Benzene (CDNB) (18). All reagents used were of analytical reagent grade. DTNB, CDNB and Thio Barbituric Acid were obtained from sigma chemicals, St.Louis; MO.

Statistical analysis

Statistical analysis between the Control Group and the Ovarian Cancer Group was performed by the independent student – t test (parametric analysis) by using the SPSS statistical package for windows, Version 15. The data were expressed as mean ± SD. $P < 0.05$ was considered as significant.

Results

The mean ± SD of erythrocyte GSH, ascorbic acid, MDA, SOD, Catalase, GP_x, plasma vitamin E and plasma GST are indicated in Table 1. There was a statistically significant increase in the erythrocyte MDA levels in patients with ovarian cancer compared to controls. The activities of the erythrocyte antioxidant enzymes SOD, GP_X and plasma GST were significantly increased in group 2 (study subjects) compared to group 1 (controls). The levels of erythrocyte GSH, ascorbic acid, plasma vitamin E and catalase activity were significantly decreased in patients with ovarian cancer compared to controls.

Discussion

The lipid peroxidation product i.e. MDA levels, were significantly increased in the erythrocytes of the patients with ovarian cancer compared to controls in this study. A significant rise in MDA levels in our patients is indicative of elevated oxidative stress. The rise in MDA could be due to increased generation of reactive oxygen species (ROS) due to the excessive oxidative damage generated in these patients. These oxygen species in turn can oxidize many other important biomolecules including membrane lipids. Similar results were obtained in the work of Nayak SB et al (19) and Kumaraguruparan et al (20) who showed an increase in MDA levels in patients with ovarian cancer. In contrast to our results, Gerber et al (21) and Saintot et al (22) reported diminished MDA levels in patients with ovarian cancer and colorectal cancer (23) and they postulated that MDA is reported to be an unstable intermediate in the peroxidation sequence of unsaturated fatty acids, which may be metabolized further or be transported.

In the present study we observed a significant decrease in the levels of non enzymatic antioxidant parameters, i.e erythrocyte glu-

Table 1 Malondialdehyde (MDA), glutathione, ascorbic acid, vitamin E, super oxide dismutase (SOD), catalase, glutathione peroxidase (GP_X) and glutathione – S – transferase in controls and patients with ovarian cancer

Variables	Control group (n = 38)	Ovarian cancer group (n = 38)	P value
Glutathione (mg/gm of hemoglobin)	18.7 ± 2.7	11.7 ± 2.9	< 0.001
Ascorbic Acid (mg/dl)	4.5 ± 1.3	4.1 ± 1.2	< 0.001
Vitamin E(µmoles/l)	7.3 ± 1.4	6.9 ± 1.4	< 0.01
MDA (nmoles/gm of hemoglobin)	5.3 ± 0.3	5.9 ± 0.6	< 0.001
SOD (U/gm of hemoglobin)	645.1 ± 40.9	672.2 ± 57.1	< 0.05
Catalase(U/gm of hemoglobin)	7.2 ± 1.4	6.4 ± 1.3	< 0.01
GP_X (U/gm of hemoglobin)	48.7 ± 1.1	50.3 ± 1.2	< 0.001
GST(µmoles/dl of plasma)	9.2 ± 0.9	13.2 ± 0.6	< 0.001

tathione (GSH), ascorbic acid and plasma vitamin E, in patients with ovarian cancer when compared to controls. Reduced glutathione, a major endogenous antioxidant, plays an important role in antioxidant defense (24). Vitamin C, a major extra cellular non enzymatic antioxidant, has a crucial role in scavenging the ROS. Vitamin E is one of the most important free radical scavenging chain-breaking antioxidant with in the biomembrane (25). The decrease in levels of these non enzymatic antioxidant parameters may be due to increased turnover, to prevent oxidative damage in these patients, suggesting increased defense against oxidant damage in ovarian cancer. Various studies have reported the decreased GSH, Ascorbic acid and Vitamin E levels in patients with ovarian cancer (26). This indicates severe damage to the antioxidant system, which is unable to control the consequences of fighting the oxidative stress.

Enzymatic antioxidants (SOD, CAT and GP_x) form the first line of the antioxidant defense mechanism to protect the organism from ROS mediated oxidative damage (27). In ovarian cancer patients erythrocyte antioxidant enzymes, i.e. SOD & GP_x activities are significantly increased. SOD is an important antioxidant enzyme having an antitoxic effect against super oxide anion. The over expression of SOD might be an adaptive response and it results in increased dismutation of superoxide to hydrogen peroxide. GP_X, an oxidative stress inducible enzyme, plays a significant role in the peroxyl scavenging mechanism and in maintaining functional integration of the cell membranes. The rise in the activity of GP_X could be due to its induction to counter the effect of increased oxidative stress. This is in accordance with the studies of Skrzydelwska et al (28). We observed a significant decrease in the activity of catalase in patients with ovarian cancer compared to controls. Catalase is the enzyme, which protects the cells from the accumulation of hydrogen peroxide by dismutating it to form water and oxygen or by using it as an oxidant in which it works as a peroxidase (29).

Glutathione – S – Transferase is a group of multifunctional proteins, which play a central role in detoxification of electrophilic chemicals and the hepatic removal of potentially harmful hydrophobic compounds from the blood (30). We observed a significant increase in the GST activity in patients with ovarian cancer compared to controls. The rise in the activity of GST could be due to its induction to counter the effect of increased oxidative stress. No significant difference in GST activity was observed in the human ovarian cancer tumor cell line and the adriamycin resistant cell line. This indicates that GST does not appear to play a role in drug resistance (31).

To conclude, this study confirmed the increased production of free radicals in ovarian cancer, which supports the existence of oxidative stress in this disease. The higher oxygen free radical production and decreased catalase activity support this finding. The increased activity of antioxidant enzymes is a compensatory activity as a response to increased oxidative stress. Our findings indicate the existence of an abnormal balance between the oxidative and protective mechanisms in these patients. The observations in the present study strongly suggest that treatment with antioxidants in the initial stages of the disease may be useful as secondary therapy to prevent oxidative damage.

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Influence of candida infection on denture stomatitis

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Objective. The aim of this study was to evaluate the influence of candida infection on denture stomatitis. **Patients and Methods.** Our study included 90 examinees of both sexes and all of them were wearers of mobile prosthetic devices for at least a year. On the basis of the anamnesis data and clinical check-up, the examinees were divided into 6 groups. In the methodology framework palate and denture plate scrapings were taken. For identification of Candida albicans and non-albicans Candida species the blastesis (germ) test, cultivation on the chromo-phyl base (Chrom agar) and the Candida assimilation test (API test) were used. **Results.** The results showed that denture stomatitis was detected in 50% of the cases. The proof of the interrelation between Candida albicans and denture stomatitis is the highly significant positive palate culture finding to Candida albicans and the denture plate culture finding. **Conclusion.** Denture stomatitis is a local determinant for stronger adherence of Candida albicans with consequent pathologic implications for the oral mucous membranes.

Key words: Candidiasis, Denture stomatitis.

Introduction

Candidiasis of oral mucosa is an opportunistic infective state caused by fungi of Candida species that contains at least 8 kinds of fungi. They are: Candida albicans, Candida glabrata, Candida tropicalis, Candida guillermondi, Candida crusei, Candida parapsilosis, Candida stelatoidea, Candida kefyr (1).

Factors of virulence that enable fungi to become pathogen are: ability of a fungus to adhere to the host tissue, production of proteolytic enzymes which help penetration into tissues, fungus hiphae - morphogenetic transformation which enables penetration and various immune-modular effects of fungal determinants which can contribute to reduction of immune system activity (2). The most common fungus causing the fungal disease is Candida albicans**.** It lives as a saprophyte on the skin and mucous membrane of the mouth, vagina and in the digestive tract.

Nevill BW et al. (3) divided the preconditions for Candida infection appearance into

two groups: 1. Factors which bring the host's immune status into disorder: blood dyscrasias, old age, radio and chemo therapy, diabetes mellitus, hypothyroidism, hypoadrenalism, hypoparathyroidism, HIV infections and so on; 2. Factors which bring oral flora and stability of oral cavity surroundings into disorder: xerostomia, antibiotic therapy, poor hygiene, malnutrition, iron and folic acid deficiency, smoking, etc. Denture stomatitis is a multi causal disease of the palate mucous membrane which is diagnosed on the basis of the following parameters: clinical observation, microbiological analysis and risk factor analysis (4). According to modified Newton's classification it is divided into 3 groups:

1. Denture stomatitis type I – localized inflammation,
2. Denture stomatitis type II – diffuse erythema without hyperplasia,
3. Denture stomatitis type III – papillary hyperplasia (5).

The etiology of denture stomatitis appearance is multi causal: ill-fitting dentures, old age, immune system disorder, smoking, wearing dentures at night, poor oral hygiene which results in denture plate plaque accumulation. It is generally confirmed that one of etiological factors is microbiological and Candida albicans presence is one of the most important factors for denture stomatitis appearance (6). The disease is manifested in palate mucous membrane changes which are the result of Candida species influence and a patient's immune reaction (7, 8).

The aim of this study was to evaluate the influence of candida infection on denture stomatitis.

Materials and Methods

Our study included 90 examinees of both sexes and different age (the average age was 59). All of them were wearers of mobile prosthetic devices for at least a year. On the basis of the anamnesis data and clinical check-up, the examinees were divided into 6 groups. Each group consisted of 15 patients: G1. Healthy patients, without denture stomatitis, G2. Healthy patients, with denture stomatitis, G3. Chronic patients, without denture stomatitis, G4. Chronic patients, with denture stomatitis, G5. Patients undergoing immunosuppressive therapy without denture stomatitis, G6. Patients undergoing immunosuppressive therapy, with denture stomatitis.

In the framework methodology palate and denture plate scrapings were taken. Native and culture findings were performed. The cultivation was on Sabouraud's dextrose agar and for identification of Candida albicans and nonalbicans Candida species, the blastesis (germ) test, cultivation on the chromo-phyl base (Chrom agar) and the Candida assimilation test (API test) were used.

Statistical analysis comprised basic statistical data: arithmetical means, standard arithmetical mean error and standard deviations, as well as the following statistical analysis tests: Levenes test, Hi-test quadrangle and T- tests for independent samples. Significance was defined as a $P < 0.05$.

Results

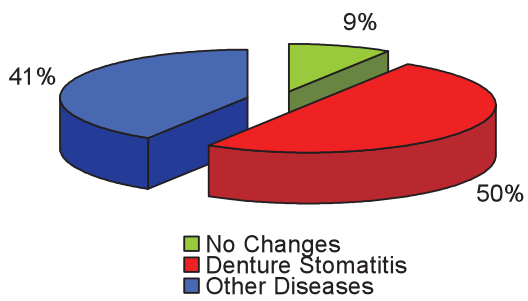

Figure 1 Percentual representation of oral finding according to categories

Denture stomatitis was detected in 50% of examinees and other diseases of oral mucous membranes in 41% of examinees.

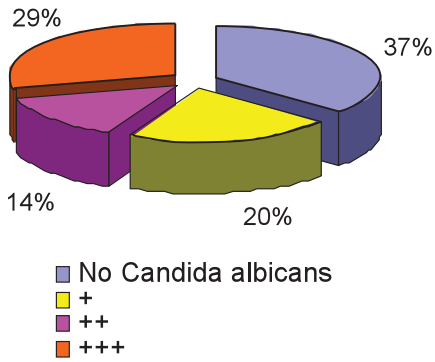

Figure 2 Native palate finding of Candida albicans

Thirty seven percent of patients did not have a positive native finding of Candida albicans and 63% of patients had a positive palate finding of Candida albicans of different intensity with domination +++ in 29% of cases.

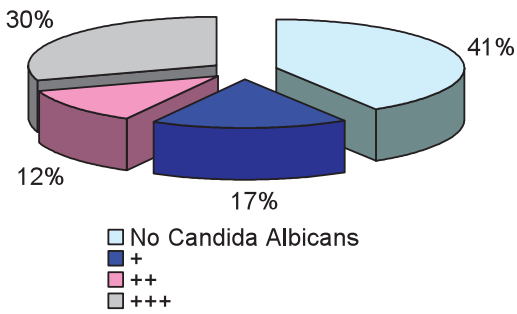

Figure 3 Native denture plate finding of Candida albicans

According to percentual presentation of native denture plate finding, 59% of patients have a positive finding of Candida albicans, of which 30% of patients have +++.

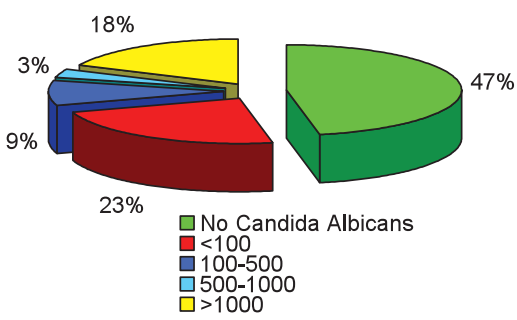

Figure 4 Palate culture finding of Candida albicans

Forty-seven percent of patients do not have positive culture finding of Candida albicans and 53% of patients have a positive culture finding. 23% of cases have less than 100 colonies and 18% have over 1000 colonies.

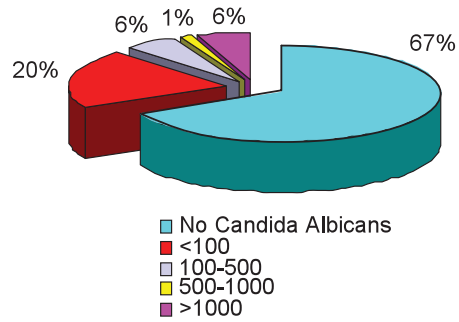

Figure 5 Denture plate culture finding plate of Candida albicans

Sixty eight percent of patients do not have positive denture plate culture findings of Candida albicans and 32% of patients have positive culture findings of Candida albicans. Groups 1, 2, 3 have a lower number of colonies in the culture finding <100 and groups 3, 4, 5 and 6 have >1000 colonies in the culture finding.

Table 1 Presence of denture stomatitis and Candida albicans culture finding from palate

Groups	Palate Culture Finding	
	n	$\bar{x} \pm$ SD
Patients without denture stomatitis	45	0.33±0.769*
Patients with denture stomatitis	45	0.80±1.236

*The levens test of variance equality: F (1.88) = 7.65, p = 0.007.

The average value of the palate culture finding to Candida albicans is statistically higher (t=2.15; df = 73.6; p = 0.04) in patients with denture stomatitis ($\bar{x}$ = 0.80) compared to the finding of patients without denture stomatitis ($\bar{x}$ = 0.33) with average difference of 0.467 (%95 CI = 0.03-0.90).

Discussion

Candidiasis of the oral mucosa is an opportunistic infection caused by fungi of the Candida species, of which the most pathogenic is Candida albicans, which selectively pathologically adheres to oral mucous membranes and causes oral diseases (7). In 50-70% of the population Candida is present as an oral commensally. (8). The results of our research showed that in the group of 90 examinees, native finding to Candida albicans was positive in 63% of patients. Denture stomatitis is an inflammatory process of the palate tegument mucous membrane caused by mobile prosthetic devices. The described prevalence is ranked from 10–75% (9). According to frequency, the microbiological aspect of Candida albicans is the most significant factor for the occurrence of denture stomatitis. The unpolished denture plate surface is several hundred times larger in micro sizes resulting in much stronger adhesion. The unpolished surface is ideal for plaque adherence and growth of micro-organisms, especially Candida albicans (10, 11).

Our results match the research results of Dorkoo et al. who isolated Candida albicans in 95 patients out of 240 who were total or partial denture plate wearers (12) and the research results of Daries et al. and associates who identified Candida albicans in 66 out of 120 patients with malignoms who also were mobile prosthetic device wearers (13). Old age is one of the risk factors connected to decreased response of cellular immunity and increased adherence of Candida albicans (14).

Our examinees, with an average age of 59, had high values of Candida albicans culture findings in patients with denture stomatitis (G4, G6). Our results match the research results of Cumming et al. who examined yeasts presence and denture stomatitis in 121 examinees in a retirement home (15). Sixty four examinees had denture stomatitis and yeasts were detected in 51 patients (78%). Poor hygiene, wearing denture plates at night, mouth breathing and smoking are local factors which led to candidiasis in patients with denture stomatitis (16, 17). Rostok et al. confirmed that Candida albicans on the denture plate surface was one of the basic causes of chronic atrophic candidiasis (11, 18).

In our research on the sample of 90 patients we also identified Candida tropicalis on the palate but only in 1.11% and on the denture plate 3.33%, while other fungi were not identified. Mosca et al. isolated Candida albicans on the palate of patients below the denture plate in 75% of cases and Candida glabrata in 16.6% of cases and also Candida dubliniensis in 8.3% of cases (19). Our results related to other species of Candida matched the mentioned research results.

Conclusion

Palate mucous membrane culture finding to Candida albicans is significantly higher in patients with denture stomatitis, and denture stomatitis is a local determinant for stronger adherence of Candida albicans with consequent pathologic implications for the oral mucous membranes.

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Residents' clinical empathy: gender and specialty comparisons - a Romanian study

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Objective. To measure and examine medical residents' (junior doctors) empathy and to compare psychiatry residents' empathy with that of other specialties. **Participants and Methods.** A translated version of Jefferson Scale of Physician Empathy for Practising Health Professionals was administered to 112 Romanian residents. **Results.** 60 residents in psychiatry and 52 in other specialities completed the questionnaire. Statistically significant differences were found between male and female counterparts, and between psychiatrists and residents in other specialities. **Conclusions.** Male doctors seemed to be less empathic compared to female ones. Psychiatry was the most empathic medical specialty.

Key Words: Empathy, Medical resident, Measurement, Psychiatry.

Introduction

One of the most studied professional attributes of physicians is empathy. Empathy plays an essential role in physician-patient relationships as it allows the patient to feel respected and appreciated. Empathy may improve the quality of the information provided to the physician during the interview, may ameliorate communication barriers between the physician and patient and ultimately may positively influence the therapeutic outcome (1-3). On the other hand, empathy can influence a physician's clinical outlook, as they may be more conscious of the bio-psychosocial, rather than the biomedical, model of disease (4).

Medical students embark on their education with idealism and enthusiasm for curing disease and helping patients. However, subsequent to this a process of cynical transformation of the medical student involving dehumanisation and de-idealisation has been described (5). At the same time, empathy appears to decline (5-7). Some of the factors thought to explain this decline are the emphasis placed on a trainee physician's emotional detachment, clinical neutrality and technical aspects of medicine, as well as the paucity of role models, life experiences and finally burnout during residency (4-8).

Little empirical evidence is available to link empathy and physician specialty. Psychiatrists, followed by physicians in general

internal medicine and paediatricians, appear to be the most empathic, while anaesthesiologists, radiologists and physicians in surgical specialties the least empathic (1, 9). There are gender differences, too, as female physicians appear to be more empathic than male ones (1, 9, 10).

This study was designed to evaluate the empathy of Romanian medical residents' according to their medical speciality and gender. We hypothesized that psychiatrists and females would be more empathic compared to other specialties and male physicians.

Participants and Methods

Participants

The study was approved by the Institutional Review Board of "Babes-Bolyai" University, Cluj-Napoca. 112 residents of various specialties from the main university centres in Romania took part in the survey. More than half (55%) were residents in psychiatry. The majority were women (74%) and were working in Bucharest and Cluj-Napoca (77%). About 59% of the respondents were in their first two years of residency.

Instruments

A back-translated version of the of the Jefferson Scale of Physician Empathy for Physicians and Health Professionals (the "HP" version) was used in this study (9). The Jefferson Scale of Physician Empathy includes 20 Likert-type items answered on a 7-point scale (half of the items are reversed scored).

Procedures

A questionnaire consisting of demographic questions and the Romanian version of the Jefferson Scale of Physician Empathy for physicians and health professionals was administered to residents of various specialities. Participation was voluntary and anonymous and the completion took place online. Invitations explaining the purpose of the study were emailed to private message boards used by medical residents. Each item had to be answered in order for the questionnaire to be validated. Two questionnaires with the same IP address were precluded due to IP address filtering. Completing the survey was considered implied consent to participate in this study.

Statistical analysis

Total scores are expressed as mean, standard deviation (SD) and range. To examine the statistical significance of the differences, the Mann-Whitney U-test for independent measures was used, with $p < 0.05$ considered significant.

Results

Internal consistency reliability of the scale was determined by Cronbach's alpha (0.84). Corrected item-total correlation ranged from 0.21 to 0.67 with a median correlation of 0.42. Items 10 and 16 had the highest correlations with the total scores (see table 1 for illustration).

Gender comparison

The mean empathy score was 113.4 (SD = 14.4; range: 43-140, skewness = -1.28 and kurtosis 4.1) in total sample; 114.9 (SD = 14.75; range 43-140) in females and 107.2 (SD = 11.5, range 78-123) in males. Total scores were not normally distributed, therefore we used the Mann-Whitney U-test to compare scores between genders. Males scored lower and the difference was significant ($z = -2.7$; $p = 0.006$).

Specialty comparison

Taking into account the medical specialty, the mean empathy score was 115.8 (SD = 15.7; range 43-140) in psychiatrists and 110.4

(SD = 12.3; range 78-134) in other specialties. Psychiatrists scored significantly higher than other specialties (z = -2.6, p=0.008). We were unable to draw conclusions for other specific specialties due to their small representation in the responding sample.

Female psychiatrists scored higher (mean = 116.7; SD = 16.0; range 43-140) than counterparts in other specialties did (mean = 111.8; SD = 12.0; range 86-134). These differences were statistically significant (z = -2.2; p = 0.025). Males scored narrowly in both groups (means around 107).

In some items, we found statistically significant differences between female and male respondents, on one hand, and between psychiatrists and other residents' answers, on the other hand (females and psychiatrists scored higher, while the others lower). Table 1 summarizes the main results.

Discussion

Our study is the first one of its kind in Romania to measure the empathy of Romanian residents. The finding that women scored higher on empathy ratings than men reaches statistical significance and is consistent with the findings of other studies (9, 11). Several explanations are offered for gender differences in empathy. Women are believed to be more receptive than men to emotional signals and to develop more care giving attitudes toward their children than men (12). There are reports that female physicians spend more time with their patients, have fewer patients and proffer more preventive and patient-oriented care (1, 9). Whilst it is unclear whether these gender differences are due to gender characteristics or due to gender role expectations, they have implications for physician selection and training. Inter-

Table 1 Comparison of scores on the Jefferson Scale of Physician Empathy of residents by gender and specialty

Number of item	Jefferson Scale of Physician Empathy	Females vs males		Psychiatrists vs other specialties	
		z	p	z	p
2	My patients feel better when I understand their feelings.	-2.429	0.015	-0.119	0.906
3	It is difficult for me to view things from my patients' perspectives	-2.108	0.035	-0.521	0.602
7	I try not to pay attention to my patients' emotions in history taking or in asking about their physical health.	-1.003	0.316	-3.586	0.000
8	Attentiveness to my patients' personal experiences does not influence treatment outcomes.	-2.866	0.004	-3.583	0.000
10	My patients value my understanding of their feelings which is therapeutic in its own right.	-2.236	0.025	-0.190	0.849
11	Patients' illnesses can be cured only by medical or surgical treatment; therefore, emotional ties to my patients do not have a significant influence on medical or surgical outcomes.	-2.486	0.013	-2.104	0.035
12	Asking patients about what is happening in their personal lives is not helpful in understanding their physical complaints.	-2.620	0.009	-2.960	0.003
13	I try to understand what is going on in my patients' minds by paying attention to their non-verbal cues and body language.	-1.299	0.194	-3.829	0.000
15	Empathy is a therapeutic skill without which success in treatment is limited.	-3.101	0.002	-2.582	0.010
16	An important component of the relationship with my patients is my understanding of their emotional status, as well as that of their families.	-3.078	0.002	-4.122	0.000

estingly, we found that female psychiatrists might be more empathetic than the other female physicians.

Junior doctors showed that they are aware of the value of empathy and that somatic complaints can be influenced by the emotional state of the patient. Residents in specialties other than psychiatry tended to pay less attention to patients' emotions, personal experiences or body language. Residents in psychiatry showed higher scores in empathy compared to internal medicine counterparts, but the differences were not significant. Nevertheless, their mean scores were lower than those reported in literature, compared to foreign medical students, residents or specialists (7, 9, 11). These differences may reflect cultural or regional aspects; the Romanian model of physician might be paying less attention to the patient-physician relationship than the North American one. Differences between specialties can be manifested in different degrees of interpersonal skills or different importance ascribed to these through the training of interpersonal skills. For example, psychiatrists say that empathy for patients is an important reason for choosing psychiatry as a career (13). On a cautionary note, our results should be seen in the context of a number of limitations: 1) the small number of surveyed subjects from a non-random sample of junior doctors; aside from psychiatry, all other specialties were underrepresented; 2) under-representation of male respondents, although this might reflect an increase in the number of females choosing to practice medicine, particularly psychiatry (12); 3) as the sample responding to the questionnaire was self-selected, it was not possible to calculate a response rate or comment on the characteristics of those who chose not to take part.

A decline in empathy during training is reported in many studies (5, 7, 10, 14). Among the hypothesises expounded for this decline are that current medical education emphasises detachment and clinical neutrality, and technological aspects of medicine predominate over humanistic ones. Other factors could be the lack of appropriate models, negative experiences during the medical education and difficulties at work (4, 10).

It is unclear if empathy is a personality state that can decline during medical education or if it can be improved by targeted educational activities. As psychiatry residents benefit from more educational programmes, targeting interpersonal skills, than other residents, we may hypothesize that empathy is amenable to change, with the direction of change more likely in a negative than in a positive direction in the absence of special programmes. These results call for further research to identify factors that contribute to changes of empathy and for the development of educational programmes designed to retain, cultivate and enhance empathy among medical residents.

In Romania, medical education emphasises clinical neutrality and detached concern, as well as biomedical models of disease. Medical universities also offer non-medical courses, usually sociology or foreign languages. Recently, behavioural courses have been introduced, but these have been taught by physicians and do not target communication skills, patient interviewing, counselling, nor identification (and solving) of psychosocial determinants that may increase the risk for disease. All of these happen while universities in other countries are striving to offer more, and better, programmes in communication and patient-centred skills in an attempt to better cultivate humanistic attitudes of future physicians.

This study represents a step towards clarification and measurement of physician empathy. This field deserves attention as empathy is important in the physician–patient relationship and has clear benefits for both patient and physician (2, 3). One should take into consideration that in the absence

of targeted programmes, empathy appears to change rather in a negative way (5-7). Further research is needed on how to promote empathy during clinical clerkships and residency.

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Placental alkaline phosphatase in the prediction of preterm delivery

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Objective. To examine the reliability of human placental alkaline phosphatase (hPlAP) in the mother's serum as a marker of premature labour among pregnant women who had no known risks for premature labour and to determine the critical value of hPlAP in pregnancies which ended up as a premature labour. **Patients and Methods.** The research was conducted in the form of a prospective study of 200 pregnant women. All the pregnant women were divided into two groups, the examinees and the control group. The value of hPlAP in serum of all pregnant women determined in the period from week 20 to 24 of gestation. > 2 median value was taken as a critical value for hPlAP . Besides descriptive statistical methods for the statistical data processing we used the χ^2 test, student t-test, Fishers test and Mann-Withneys test, logistic regression. **Results.** The number of premature labours in the examined group was 17 (11.3%), in the control group 22 (44%). The probability of premature labour is 6.1 times higher in the control group in relation to the examined group. The mean value of hPlAP in the examined group was 608.2 but in the control group 1115.6. The mean value of hPlAP in the pregnant women who gave birth prematurely was 1195 but in those who gave birth on time 632.2. There was a statistical significant difference in mean values of hPlAP. **Conclusios.** hPlAP can be used as a reliable marker of idiopathic premature labour. hPlAP values connected with the development of premature labour is 990 mU/l.

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Key words: Preterm labour, Placental alkaline phosphatase, Prediction.

Introduction

According to the World Health Organisation (WHO) and the American Pediatrics Academy each delivery which occurs before 37 gestational weeks is considered a preterm delivery (1). The best and most effective prevention of preterm delivery incidence is early identification of pregnant women who belong to a group of high risk (2). Meth-

ods of identification include clinical and biochemical markers of preterm delivery. Various pieces of research have pointed out that human placental alkaline phosphatase (hPlAP) can be used as a possible marker of idiopathic preterm delivery (3).

The placental specific isozyme of Alkaline Phosphatase (PlAP) is found in the trophoblast cells of a normal human mature placenta (4). Human placental alkaline phosphatase (hPlAP) are polymorphic and heat-stabile enzymes. They are localised in the apical and basal cells of the syncytiotrophoblast plasma membrane and at the surface of cytotrophoblast chorionic villuses. Human phosphatase is a sialoglycoprotein in which glycosyl phosphoinositol is situated consisting of two identical subunits. There are high levels of this enzime in the trophoblasts of the placenta, while it can also be traced in the lungs, endocervix and Fallopian tubes. Its creation starts at about the seventh week of gestation (5). Elevated placental alkaline phosphatase levels may signal an increased risk of preterm delivery. Placental alkaline phosphatase, a glycoprotein found in maternal serum, increases with gestational age and normally peaks at term. The odds of delivering preterm were determined greater among mothers with higher placental alkaline phosphatase levels. Elevated mid-trimester serum levels among these mothers may indicate a breakdown of the foetal membranes (6).

The aim of this study was: to examine the reliability of hPlAP in mothers serum as a marker for preterm delivery with women who do not have any of the proved risks which can cause preterm delivery, and determine the value of hPlAP which correlates with preterm birth incidence.

Patients and Methods

The study was conducted as a prospective study and included 200 pregnant women. Correct gestational age was determined according to the last menstrual cycle and ultrasound biometry during the first trimester. We determined gestational age at the time of delivery in pregnant women who delivered before 37 gestational weeks and the number of preterm deliveries. Pregnant women were divided into examinees and a control group. 150 pregnant women who were regularly controlled in ante-natal clinics formed the examinee group. The control group consisted of 50 pregnant women who were admitted to the Gynaecology and Obstetrics Clinic in Tuzla. They were admitted for a symptoms of preterm labour. In both groups of pregnant women none of the well known risk factors for preterm delivery incidence were present. The standards used for selection of women for the control group were: a tocolytics index less than 4, intact membranes and absence of contraindications for tocolytics therapy.

The value of hPlAP in serum was determined in all women in the period of 20 to 24 weeks gestation. In the course of pregnancy, the incidence of preterm birth was monitored. hPlAP was determined by the Elisa method using monoclonal antibodies with the help of Innotest hPlAP. Innotest is manufactured by Innogenetics, Belgium. The value > 2 was taken as the critical value (a value greater than two median values). This value is expressed in mU/l.

During data processing descriptive statistical methods were used, mean values, standard deviation and the control group were compared by the x^2 test, student t-test, Fishers test and Mann-Whitney's test. Logistic regression was used to determine whether placental alkaline phosphatase was associated with preterm birth.

Results

The examined group consisted of 150 (100%) women, 17 (11.3%) had preterm delivery

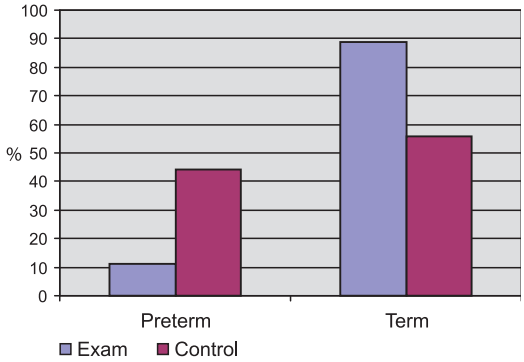

Figure 1 Number of preterm deliveries in the examined and control groups

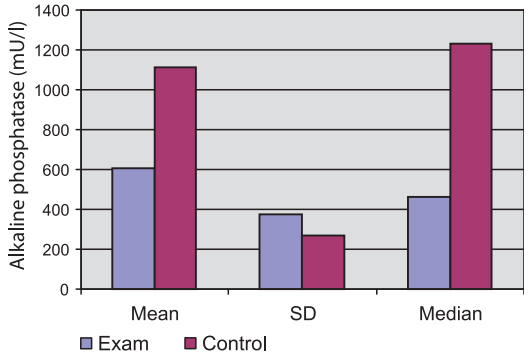

Figure 2 Value of human placental alkaline phosphatase in examined and control groups

Table 1 Gestational age at preterm delivery in examined and control groups

Weeks of gestation	Examined group		Control group	
	N	%	N	%
26- 30	–	–	4	8
31-34	2	1.3	8	16
35-37	15	1.0	10	20
Total	17	11.3	22	44

$\chi^2 = 5.9$; $p = 0.015$

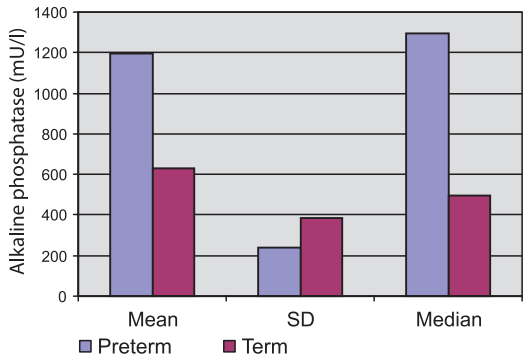

Figure 3 Value of human placental alkaline phosphatase in preterm delivery and term delivery in both groups

while the number of term deliveries was 133 (88.6%). The control group consisted of 50 (100%) women, 22 (44%) had preterm delivery while the number of term deliveries was 28 (56%). The probability of preterm delivery incidence was 6.1 times greater in the control than in examined group (95% CI: 2.9-13.1), $x^2 = 25.5$, $p < 0.0001$.

In the examined group there were 2 deliveries (1.3%) before 34 weeks gestation, in the control group 12 deliveries (24%) in the same period of gestation (< 34 weeks gestation). The probability of preterm delivery was 9.0 times greater in the control than in the examined group for gestational age < 34 weeks gestation (95% CI: 1.4-94.2).

The mean value of hPlAP in the control group was 1115.6 mU/l with standard deviation 377.8 and median value 460. Mean values were tested by t-test. It was shown that there was a statistically significant difference between the mean values of hPlAP in the examined and control groups ($t = 8.7$; $df = 199$; $p < 0.0001$) (Figure 2).

Mean value of hPlAP in preterm delivery was 1195 mU/l with standard deviation 240 and median value 1295. Mean values were tested by t-test. It was shown that there was a statistically significant difference between mean values of hPlAP in preterm delivery and term delivery ($t = 8.2$; $df = 199$; $p < 0.0001$) (Figure 3).

Following Mayer et al (7) median values calculated were used for concentration of hPlAP in preterm and term deliveries. Concentrations of hPlAP greater than two median values and their incidences in term delivery were also calculated. It was proved

by x^2 test that there is a connection between preterm delivery incidence and hPlAP concentration (x^2 = 438; p = 0.0001) (Table 2). The Fisher exact test brought us to the same conclusion. While calculating the relation between the probability of preterm delivery and term delivery, the conclusion was reached that the probability of preterm delivery is 12.2 times greater with increased values of hPlAP. Regarding the sensitivity and specific quality of hPlAP, it was concluded that the sensitivity of the test was 71.8% and the specific quality 82.7%.

Table 2 Median value of concentration of human placental alkaline phosphatase (hPlAP) <2 (median) and >2 (median) in preterm and term delivery in both groups

Delivery	Median hPlAP < 2	Median hPlAP > 2
Preterm	11 (5.5%)	28 (14%)
Term	133 (66.5%)	28 (14%)

x^2 = 43,8; p = 0.0001

Discussion

Preterm delivery is the leading cause of mortality in newborns. About 65 to 70 % of foetus deaths and the incidence of early neonatal death are in children born before the end of the 37th week of gestation with body weight less than 2500 grams (8). Women with high levels of hPlAP in the midtrimester have a higher risk for preterm delivery incidence. The biological mechanisms involved in this process are unclear. Necrosis, rupture and other damage of the chorionic villuses, infarction of the placenta or ablation of the placentae can increase the levels of alkaline phosphatase of the placenta in the serum (9). This is found in women who suffer from preterm delivery and it is a clear indication of integrity disorders of the foetus membranes. Many studies have indicated the relationship between increased levels of serum thermostabile alkaline phosphatase and low birth weight and placenta insufficiency (10). The smallest value in the control group was 730 mU/l while in the experimental group it was 150 mU/l. The highest value in the control and experimental groups was 1375 mU/l.

Thermostable hPlAP in women who have normal course of pregnancy is an indicator of placenta function and indirectly an indicator of the condition of the foetus (11). With as the pregnancy progresses, the statistical and significant growth of hPlAP in obvious. There are individual variations in hPlAP at some gestational periods in different women. It was found that the mean value of hPlAP was 1280 mU/l within the first eight weeks of pregnancy. In 28 (56%) women who suffered from preterm delivery, the value of hPlAP > 2 was found, 11 (22%) women had values < 2. The probability of preterm delivery where hPlAP value is > 2, is 12.2 times greater than term delivery. In this study a much higher percentage of pregnant women had values > 2 compared to the study by Meyers et al where 33% of women had value > 2. According to the study by that author the probability of a preterm delivery is 2.9 times greater than term delivery if the value of hPlAP is increased. High specificity means that patients who have high values of hPlAP will most likely suffer from preterm delivery. The possibility of preterm delivery increases with the increase of alkaline phosphatase level (12).

Conclusions

According to the obtained results the following conclusions were made:

- Human placental alkaline phosphtase (hPlAP) is a reliable marker of preterm delivery with patient who do not have any proven risk of preterm delivery and the marginal value of hPlAP connected with preterm delivery is 990 mU/l.

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Idiopathic thrombocytopenic purpura in children

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Idiopathic thrombocytopenic purpura (ITP) is the most common acquired bleeding disorder in childhood. ITP is characterized by a low circulating platelet count caused principally by destruction of antibody-coated platelets in the reticuloendothelial system. It can be classified into two major forms, acute and chronic. Acute ITP is usually a benign self-limiting condition with a high probability of spontaneous recovery. Rates of 80% complete remission can be achieved regardless of the treatment. Persistence of thrombocytopenia for more than 6 months defines the chronic form of the disorder, and is seen in approximately 20% of children. Children with chronic ITP also have a good prognosis, with up to 80% remissions over a period of years following diagnosis. The variability of clinical course makes the decision of whether and how to treat difficult. Most children with ITP have mild bleeding symptoms and require no therapy. The commonly used regimens include corticosteroids and intravenous immunoglobulins. So far, there is no evidence that initial therapy can prevent major bleeding or a chronic course of the disease. ITP in childhood remains a disorder with many unsolved questions regarding pathophysiology, diagnostic approach and therapeutic decisions. Large prospective clinical trials with long-term follow-up are needed to define which subgroup of children with ITP should be treated with platelet-enhancing therapy.

Key words: Idiopathic thrombocytopenic purpura, Child.

Introduction

In 1951 William Harrington published the results of his study in which he injected plasma of patients with thrombocytopenic purpura into healthy volunteers (1). They developed a rapid and profound, but transient decrease of platelet counts. Dr. Harrington repeated this experiment on himself as many as 35 times over 2 years. He postulated the presence of an "antiplatelet factor" that subsequently was confirmed as an immunoglobulin (2). Since this human experiment, innumerable articles have been published on idiopathic thrombocytopenic purpura. Although the autoimmune nature of the disease has been appreciated for over 50 years, many aspects of the pathogenesis

have been unanswered and the treatment has remained opportunistic and empirical.

Background

Idiopathic or immune thrombocytopenic purpura (ITP) is the most common acquired bleeding disorder and the most common thrombocytopenia of childhood. The annual incidence is 5 out of 100,000 children (3). ITP is an autoimmune disorder characterized by premature destruction of antibody-sensitized platelets by phagocytic cells in the reticuloendothelial system, mainly in the spleen (4). ITP can be classified based on the duration of the illness into two major forms, acute and chronic. Acute ITP is usually a benign, self-limiting condition presenting in children of either sex between the ages of 2 and 10 years. Often a viral infection or immunization precedes the onset of acute ITP by some days or weeks. Persistence of thrombocytopenia, which is generally defined as a platelet count of less than 150 x 10^9/l, beyond the arbitrary endpoint of 6 months after the initial presentation, defines chronic ITP. Chronic ITP is seen in approximately 20% of children. Factors associated with the development of chronic ITP include age older than 10 years, female gender, and insidious onset. A small number of chronic cases exhibit an intermittent pattern of thrombocytopenia, and are classified as having relapsing or recurrent ITP (5, 6).

Children in whom ITP is diagnosed have an excellent prognosis. Approximately 80% of patients will recover a normal platelet count by 6 months after diagnosis, following pharmacotherapy or observation alone. Even children with the chronic form of the disease have a high potential of spontaneous or drug-induced remission, at a rate of 30 to 80%, which is much higher than in adults (7).

It is also important to distinguish these primary disorders from secondary causes of thrombocytopenia by identification of associated symptoms (Table 1).

Table 1 Secondary causes of ITP in children

Secondary causes of ITP in children
Systemic lupus erythematosus
Immunodeficiency syndromes
Bone marrow failure syndromes
Lymphoproliferative disorders
Myelodysplastic syndromes
Human immunodeficiency virus-associated thrombocytopenia
Drug-induced thrombocytopenia
Alloimmune thrombocytopenia
Congenital/hereditary nonimmune thrombocytopenia

Pathogenesis

Platelets are produced by megakaryocytes in the bone marrow, and have an average life span of 10 days. It was originally thought that platelets were released by shedding from the outer surface of the megakaryocyte, but actually the entire megakaryocyte cytoplasm fragments into platelets, leaving behind a nucleus to be removed by marrow macrophages. Normal marrow contains 6 x 10^6 megakaryocytes per kilogram body weight, with each megakaryocyte releasing up to 1000 platelets. The normal platelet count ranges from 150 to 400 x 10^9/l (8). The spleen continually but transiently sequesters about a third of circulating platelets. Platelets have several growth regulators, including IL-3, IL-6, IL-11, stem cell factor, and erythropoietin. The most important regulatory growth factor is thrombopoietin, a polypeptide that stimulates platelet production following binding to a specific receptor on hematopoietic stem cells, megakaryocytic precursors and megakaryocytes. Thrombopoietin is cleared by binding to its receptor on platelets, and is thought to be produced constitutively (9).

Platelets have a variety of membrane receptors that allow them to interact with different substrates and with each other. The major function of platelets is to maintain primary hemostasis where they lie at the endothelial cell junction, and adhere to the exposed subendothelium of a damaged blood vessel. A platelet count of at least 7 x 10^9/l is necessary to support the vascular integrity. Platelets are involved in every step of the hemostatic process. The essential step of the platelet adherence is the interaction of glycoprotein receptors (GP1b) on the platelet surface with Von Willebrand multimers and exposed collagen microfibrils from the subendothelium. Platelets that have undergone adhesion secrete agonists, thus leading to aggregation of more platelets to form an enlarging platelet plug. With aggregation there is a release of hemostatically active substances from platelet granula, including vasoactive amines, adenine nucleotides, platelet-derived growth factor, beta thromboglobulin, and platelet factor 4. This results in amplification and recruitment of additional platelets, donating their membrane phospholipids for the activation of coagulation factors, thus facilitating thrombin generation and formation of fibrin clot (8, 9).

A key element in the pathogenesis of ITP is loss of self tolerance, leading to the production of autoantibodies against platelet membrane antigens. Children with ITP have polyclonal and monoclonal antibodies with specificity against platelet-specific antigens, in particular glycoproteins IIb/IIIa and Ib/IX (10). These autoantibodies are predominantly IgG, but IgM and IgA types have also been described (11). Reticuloendothelial cells, mainly macrophages found principally in the spleen, bearing receptors for the Fc portion of IgG, clear platelets coated with antibodies from the circulation (12).

For more than 50 years, the only underlying problem in ITP has been recognized as autoimmune platelet destruction, and it was assumed that thrombopoiesis was maximized in response to increased platelet clearance. It has been shown that impaired platelet production also is important in many cases, reflecting the inhibitory effect of platelet antibodies on megakaryopoiesis. Thrombopoietin levels in ITP patients are normal or slightly elevated in contrast to high levels found in thrombocytopenia due to bone marrow failure (9, 12).

The essence of the problem is why children with ITP develop an abnormal immune response. Almost two thirds of the patients have a recent history of viral illness, and it has been postulated that molecular mimicry between viral and self-antigens could initiate autoimmunity. Alternatively, previously suppressed "naturally occurring" autoreactive antibodies might emerge that have escaped natural immune suppression called peripheral tolerance (4). As a part of the platelet destructive process in ITP, cryptic epitopes from platelet antigens are exposed, leading to the formation of new platelet-specific antibodies. It is increasingly apparent that cellular immune mechanisms play a pivotal role in ITP. The production of antiplatelet antibodies by B cells requires antigen-specific T helper (Th)-cells. Ongoing interaction between T cells and B cells is necessary to maintain active platelet autoimmunity. It is also possible that cytotoxic T cells play a role in the destruction of platelets (13, 14).

A number of investigators have studied the pattern of cytokine production in ITP. While there are conflicting reports, most results have shown elevated levels of interleukin-2 and interferon-gamma, favoring prevalence of Th1 subtype cells (12).

Genetic factors have been also proposed to play a role in the development of ITP. Studies have failed to detect linkage to particular HLA genotypes. It has been postulated that polymorphisms in Fcγ receptors of spleen macrophages, leading to functional differences in the ability to bind immuno-

globulin G, may influence the development of ITP (15).

Clinical presentation

The typical presentation of acute ITP is the abrupt onset of skin bleeding in a previously healthy child. There is usually a history of recovery from a recent infectious illness, most often a viral upper respiratory tract infection or gastroenteritis. A seasonal fluctuation, with the peak in the winter and spring months, can be seen. In a minority of cases, ITP follows a specific viral infection, such as Epstein-Barr virus, cytomegalovirus, HIV, rubella, parvovirus, varicella-zoster virus, and hepatitis A, B, C, although there is no correlation between the severity of the viral illness and the degree of thrombocytopenia at presentation. ITP may also be seen following recent immunization with a live virus vaccine, such as the measles, mumps, and rubella vaccine.

More than 95% of patients present with non-palpable hemorrhagic skin lesions (9, 16). Depending on the size, skin hemorrhages are traditionally classified as petechiae (less than 2 mm in the greatest diameter), purpura (2 mm to 1 cm), and ecchymoses (more than 1 cm) (17). In addition to bruising, less than one third of children with ITP present with epistaxis and oral mucosal bleeding. Hematuria, hematochesia, or melena is observed in less than 10 percent of patients. Menometrorrhagia may be seen in adolescent females. Conjunctival and retinal hemorrhages occur infrequently. A slightly palpable spleen is seen in 10 percent of patients.

Although more than half of children with ITP have very low platelet counts, bleeding episodes are less severe than in patients with hypoproductive thrombocytopenia. This finding is consistent with the presence of young, large, hemostatically effective circulating platelets (18). Hence, the physical examination of a child with ITP should be essentially normal aside from evidence of purpuric rash. Malaise, fever, bone or joint pain, remarkable lymphadenopathy, or hepatosplenomegaly are very uncommon findings and should raise strong suspicious of another etiology, such as acute leukemia (5, 12).

Children with ITP usually present between the ages of 2 and 10 years, with a peak incidence at 2 to 5 years. Both sexes are affected equally. Patients who are younger than 2 years or older than 10 years are more likely to develop chronic ITP in combination with some other autoimmune disorder.

Typically, in an untreated child, the bleeding resolves and the platelet count returns to normal in approximately 1 to 3 weeks. About half of the patients achieve a normal platelet count in 4 to 8 weeks, while ITP resolves in two thirds of cases by 3 months. Complete remission, defined as a platelet count greater than 150 x 10^9/l within 6 months of initial diagnosis and without need for ongoing platelet-enhancing therapy, occurs in 80% of patients. This excellent outcome seems independent of any treatment strategy (19, 20).

Chronic ITP includes a small number of children who continue to have persistent thrombocytopenia (platelet count of less than 150 x 10^9/L) beyond 6 months from initial presentation. Although there are no specific predictors for chronic ITP, it occurs more commonly in females aged over 10 years at diagnosis, is usually insidious at onset, and commonly lacking infectious prodroms. Unlike acute ITP, it does not show a seasonal predilection. Children often present with a higher platelet count compared with acute ITP. Most of them are either asymptomatic or present with a history of easy bruising or mucosal bleeding for several months' duration. In this group of patients additional testing should be considered, as secondary causes of thrombocytopenia are more likely (5, 21). Only 10 do 20% of children with acute onset of ITP show a chronic

course according to the definition. Of these, however, over a period of months to many years, 30 to 80% recover completely, regardless of prior treatment (7). According to this observation, chronic ITP in childhood is very rare, and may be subdivided into a mild, therapy-independent form (platelet count > 20 x 10^9/l) and a severe, therapy-dependent form (ongoing bleeding that requires therapy, platelet count usually < 20 x 10^9/l) with an annual incidence of 1 per 2,5 million (22, 23).

The gravest and most feared complication of ITP is intracranial hemorrhage (ICH). Its incidence is fortunately lower than initially thought, and occurs at a rate of 0.1 to 1%. Half of the bleedings occurs in the first 1 to 2 months after initial diagnosis, but are described at any time during the course of the illness, when only a small fraction of the patients are still severely thrombocytopenic. Although the incidence of ICH is extremely low, the mortality rate is significant, with 50% of early occurring hemorrhages being fatal (24). There are no defined ways to predict which patients will develop ICH. Important factors associated with a higher risk of ICH include head trauma, arteriovenous malformation, and use of antiplatelet drugs, such as aspirin, in a child with very low platelet counts (< 10 x 10^9/l). These patients need to be identified early and treated aggressively (25, 26).

Bleeding severity in children with ITP is usually commensurate with the degree of thrombocytopenia, but some patients have minimal or no hemorrhage despite a very low platelet count. Several attempts have been undertaken to establish a scoring system for defining the extent of bleeding in order to standardize therapeutic decisions. Descriptive methods have been employed first. The term "dry" purpura has been used to define cutaneous hemorrhage alone, whereas "wet" purpura signifies active mucous membrane hemorrhage, which is considered susceptible to major bleeding. Bolton-Meggs and Moon arbitrarily divided bleeding signs into four categories: none, mild, moderate, and severe (Table 2) (27). Recently, Buchanan developed a scoring system by measuring signs and symptoms of bleeding on the basis of physical examination and history of new bleeding during the previous 24 hours (Table 3) (28). Bleeding severity is measured on a 5-point scale, and assessed in the skin, from the nose, from the mouth, and globally. The global score encompasses other sites, including menorrhagia, gastrointestinal hemorrhage, and internal bleeding (28, 29).

Table 2 Classification of childhood ITP on the basis of clinical symptoms*

None	No symptoms beyond low platelet count
Mild	Bruising and petechiae
	Occasional minor epistaxis
	Very little or no interference with daily living
Moderate	More severe skin manifestations with some mucosal lesions
Severe	More troublesome epistaxis and menorrhagia
	Bleeding episodes (epistaxis, melena, and/or menorrhagia) requiring hospital admission and/or blood transfusion
	Serious interference with quality of life

*From: Bolton-Maggs PHB, Moon I. Assessment of UK practice for management of acute childhood idiopathic thrombocytopenic purpura against published guidelines. Lancet. 1997;350:620-23.

Table 3 Clinical grading of hemorrhage in childhood ITP based upon history (prior 24 hours) and physical examination*

	Grade 0 (None)	Grade 1 (Minor)	Grade 2 (Mild)	Grade 3 (Moderate)	Grade 4 (Severe)	Grade 5 (Life-threatening or Fatal)
Skin	–	Possibly a few new petechiae and bruises	Definitely new petechiae and bruises	Numerous petechiae and bruises	Extensive petechiae and bruises	–
Epistaxis	–	Blood in nares or on pillow	Active bleeding ≤15 minutes	Active bleeding >15 minutes	Repeated or continuous bleeding	–
Oral	–	Petechiae on palate	Submucosal blood "blisters"; no active bleeding	Intermittent active bleeding	Continuous bleeding	–
Overall	–	Minor or mild skin bleeding; no mucosal hemorrhage	Moderate or severe skin bleeding; no mucosal hemorrhage	Mucosal bleeding not requiring medical attention	Mucosal bleeding or suspected internal hemorrhage requiring medical attention	Documented CNS or life-threatening or fatal hemorrhage in any site

*From: Buchanan GR. Bleeding signs in children with idiopathic thrombocytopenic purpura. J Pediatr Hematol Oncol. 2003;25(Suppl 1):S42-6.

Diagnosis

The diagnosis of childhood ITP is based principally on the exclusion of other causes of thrombocytopenia. Typically, the child with ITP is otherwise healthy, looks well, and presents with spontaneous sudden appearance of purpura and/or bruising. A comprehensive history includes questions regarding recent infections, immunization, use of medications, and personal and family history of bleeding tendency. The physical examination is normal besides purpura. A complete blood count shows isolated thrombocytopenia. Roughly 80% of children present with platelet counts less than 20 x 10^9/l, and often less than 10. Mild anemia with normal red blood cell indices can be seen in as many as 15% of children, indicating a preceding bleeding. The leukocyte count is usually normal, but in some cases may be altered due to concomitant or recent viral or bacterial infection (30). The careful examination of the peripheral blood smear is of utmost importance, excluding the existence of pseudothrombocytopenia caused by platelet clumping, and confirming the normal morphology of all cell lines. Large platelet forms can be seen, particularly when symptoms have been present several days or longer. Coagulation studies are not warranted. Prothrombin time and partial tromboplastin time are invariably normal. Bleeding time, which is a direct test of platelet function, is almost always prolonged but is often shorter than expected for the degree of thrombocytopenia, suggesting that platelet function is normal or increased in many patients with ITP.

In summary, if isolated thrombocytopenia is present and there are no atypical findings that are uncommon in ITP or suggest other etiology, complete blood count and careful film examination by an experienced morphologist should be done, but no further diagnostic tests are indicated. On the other hand, abnormal red blood cell or leukocyte counts or abnormal morphology than cannot be explained easily (e.g. anemia secondary to mucosal bleeding; leucopenia in cases of concomitant viral infection; atypical

lymphocytosis in cases of infectious mononucleosis) should prompt further diagnostic evaluation. The one exception is mild eosinophilia, which is a common finding (9, 12).

Serologic testing for antiplatelet antibodies is not recommended as a part of the routine diagnostic strategy. Although a variety of tests are available, there is as yet no reliable test that has a sufficiently high sensitivity, specificity and reproducibility. Indirect tests that detect free antiplatelet antibodies in the plasma are inferior to direct tests that detect platelet-bound antibodies. Clinicians should be aware that negative test results do not exclude the diagnosis of ITP. Positive test results may lead to inappropriate treatment. Positive testing is described in benign disorders, such as gestational thrombocytopenia, that requires no treatment, or critical disorders, such as thrombotic thrombocytopenic purpura, that requires urgent plasma exchange. Besides, false-positive tests may simply reflect the increased alpha-granule immunoglobulin G present in children (31).

The investigation of proportion of reticulated platelets (young platelets with high RNA content) by fluorescence activated cell sorter (FACS) can help to distinguish between short platelet survival and failure of production, but is not necessary.

Measurement of thrombopoietin plasma levels can be informative in complex cases of thrombocytopenia, and can be useful in distinguishing between reduced production of platelets (high thrombopoietin level) and increased destruction of platelets (normal level). This assay is not recommended as a part of the routine investigation of ITP (32).

Bone marrow aspirate, if performed, typically shows a normal to increased number of megakaryocytes, many of which are immature. An increase in the number of marrow eosinophils and their precursors is present in some cases (33).

It is still a subject of debate whether bone marrow should be examined routinely in children who have suspected ITP. The consensus in the United States, as well as most European countries, is that bone marrow aspiration does not need to be performed in children with a typical clinical presentation and isolated thrombocytopenia, if an observation alone or immunoglobulin therapy has been chosen. The issue of whether bone marrow examination should be done in children before corticosteroid therapy remains still unsolved, but the majority of pediatric hematologists perform a bone marrow aspiration before initiating steroids to exclude the possibility of "masking" acute leukemia. Bone marrow aspirate is necessary in all children with acute ITP and atypical clinical (e.g. hepatosplenomegaly, lymphadenopathy) or laboratory features at diagnosis, as well as in children who fail to achieve any response to initial treatment, or have a chronic course of the disease.

Differential diagnosis

The differential diagnosis of thrombocytopenia in the pediatric population is very broad. Table 4 lists selected causes of thrombocytopenia, and clinical and laboratory features that distinguish them from ITP (9). It is worth emphasizing again that the diagnosis of ITP is based on the presence of an isolated thrombocytopenia in a well-appearing child, and in the absence of atypical clinical and laboratory features.

A complete past medical history and family history is very important. Hereditary thrombocytopenias, including von Willebrand disease type 2B and platelet-type or pseudo-von Willebrand disease may mimic the presentation of ITP, but other family members are often affected, and a larger degree of mucocutaneous bleeding present given a platelet count. History of recurrent infections may lead to the diagnosis of congenital or acquired immunodeficiencies. Thrombocytopenia is a hallmark of Wis-

Table 4 Differential diagnosis of thrombocytopenia in childhood*

Disorder	Clinical features	Laboratory features	Diagnosis confirmation
Immune thrombocytopenic purpura	Petechiae, ecchymoses Rare mucosal bleeding	Thrombocytopenia Rest of CBC normal	By exclusion of other disorders
Drug-induced thrombocytopenia	Petechiae, ecchymoses History of recent exposure to drug	Thrombocytopenia Rest of CBC normal	By measuring drug dependent antibodies When drug withdrawn, thrombocytopenia resolves
Thrombocytopenia absent radius syndrome (TAR)	Diagnosed during infancy Skeletal abnormalities (radial hypoplasia, abnormal thumb)	Thrombocytopenia	Clinical plus laboratory observations
Acquired aplastic anemia	Related to the severity of pancytopenia (e.g. pallor, petechiae, active bleeding)	Generalized pancytopenia	Bone marrow aspiration
Fanconi anemia	Short stature, thumb and other skeletal abnormalities	Other cytopenias may be present. Macrocytes on blood smear	Increased chromosomal fragility when cells exposed to diepoxybutane Genetic analysis
Von Willebrand disease type 2B	Mucosal bleeding Family history of thrombocytopenia	Decreased levels of von Willebrand factor	Ristocetin-induced platelet aggregation Genetic analysis
Bernard – Soulier syndrome	Family history of thrombocytopenia (autosomal recessive)	Mild thrombocytopenia Large platelets	Flow cytometry of platelets (decreased GP Ibα-V-IX complex expression)
Giant platelet syndromes (May – Hegglin, Hermansky – Pudlak, Sebastian)	Family history of thrombocytopenia (autosomal dominant) Renal disease Deafness	Giant platelets (size of red cell) seen on the blood smear Some syndromes include neutrophil inclusions	Electron microscopy of platelets
Wiskott-Aldrich syndrome	Males (X linked) Usually signs of immunodeficiency Recurrent otitis media, eczema	Small platelets seen on smear Low MPV Absent or decreased isohemagglutinins	Abnormal CD43 expression Genetic analysis
Acute leukemia	Usually lymphadenopathy, splenomegaly, hepatomegaly	Other blood counts affected Leukocytosis and anemia	Bone marrow aspiration, cytogenetic analysis
Systemic lupus erythematosus	Usually older children > 10 years Clinical criteria for lupus (e.g. fine hair, rash, joint swelling, etc)	Anemia and leucopenia can be present Elevated ANA, anti-double stranded DNA	Clinical plus laboratory data
Hemolytic uremic syndrome	History of bloody diarrhea Acute renal failure	Elevated BUN and creatinine Schistocytes on blood smear Escherichia coli 0157:H7 in stool	Clinical plus laboratory data

*From: Di Paola JA, Buchanan GR. Immune thrombocytopenic purpura. Pediatr Clin N Am. 2002;49:911-28.

kott-Aldrich syndrome, but has associated features of eczema, recurrent infections, and a propensity to develop autoimmune disorders. It usually presents in the first months of life, and there is a predisposition to significant bleeding out of proportion to the degree of thrombocytopenia. Congenital amegakaryocytic thrombocytopenia is a bone marrow failure syndrome that presents with severe thrombocytopenia in the neonatal period. HIV-associated thrombocytopenia should be considered in a child with a family or transfusion history compatible with this diagnosis. Drug-induced thrombocytopenia is uncommon, but can occur in children who have had a recent illness and have completed or are completing a course of medications such as penicillin, sulphonamides, or quinidine.

Besides a history, it is essential to make a thorough physical examination of a child with thrombocytopenia. Special attention should be given to the presence of skeletal malformations or short statue, which is found in patients with Fanconi anemia and thrombocytopenia-absent radii syndrome. Cutaneous rash and joint swelling might be suspicious of a more severe autoimmune disorder like systemic lupus erythematosus that affects usually children older than 10 years. The presence of hepatosplenomegaly, lymphadenopathy, or bone pain in an ill-appearing child is a characteristic of hematological malignancies.

Finally, a careful assessment of a peripheral blood smear by an experienced hematologist cannot be overemphasized. In inherited thrombocytopenias platelet morphology and platelet size are very useful in making the proper diagnosis. Bernard-Soulier syndrome is characterized by abnormal large platelets and a significant degree of bleeding. May-Hegglin anomaly is another syndrome associated with giant platelets and variable thrombocytopenia. One of its distinguishing features is large inclusions in granulocytes and monocytes known as Dohle bodies. Thrombocytopenic purpura and hemolytic uremic syndrome are characterized by thrombocytopenia, microangiopathic hemolysis, and organ dysfunction. The blood smear reveals fragmented cells, schistocytes, and microspherocytes. The presence of blast cells indicates hematological malignancy and should prompt bone marrow aspiration (9, 34).

Treatment

There is a consensus that childhood acute ITP is generally a short benign self-limited disorder that, in the majority of cases, requires minimal or no therapy. The natural history of the disease is that 80% of cases recover within a few months of presentation with or without therapy. It is recognized that drug therapy does not alter the clinical course of the disease but may shorten the period of profound thrombocytopenia. Therefore intervention, if any, should be directed at early control of active bleeding. Standard supportive care that is important in the management of a child with very low platelet counts includes avoidance of medications with antiplatelet or anticoagulant activity. If possible, the child's physical activity should be limited as long as the platelet count is less than 20 x 10^9/L, there is an excessive bruising or new petechiae, or both. Contact sports should be avoided while the platelet count is less than 50 x 10^9/l. In active young children with marked thrombocytopenia, where limiting their activities is not feasible, protective headgear may be helpful, as well as for toddlers lining their crib with protective padding.

Pharmacotherapy or observation only of a child with acute ITP has been discussed for years, and has divided pediatric hematologists between so called interventionists and non-interventionists (5, 35). Interventionists advocate the use of drug therapy in all children with very low platelet counts to prevent severe bleeding. Non-interventionists follow the "watchful waiting" method,

involving careful observation and reassurance of the patient and parents, and arguing that therapy, often causing undesirable side effects, has not proven to prevent intracranial hemorrhage. Several different guidelines for the diagnosis and management of ITP in all age groups have been published, and are still a subject of debate. The American Society of Hematology (ASH) practice guidelines recommend that children with ITP and platelet counts less than 20 x 10^9/l and significant mucosal bleeding, or those with platelet counts less than 10 x 10^9/l and minor purpura, be treated with specific regimens of IVIG or oral prednisone (36). By contrast, recommendations from the British Paediatric Haematology Working Group state that treatment of children with ITP should be decided on the basis of clinical symptoms, not on the platelet count alone. Pharmacotherapy is reserved for children who have an overt hemorrhage and the platelet count less than 20 x 10^9/l, or those who have an organ- or life-threatening bleeding irrespective of the platelet count (37).

Despite existing guidelines, the decision of when to treat, what treatment to use and the need for hospitalization is based more on opinion than evidence. Initial treatment options for childhood ITP include steroids, intravenous immunoglobulin, and, for children who are Rh positive, anti-Rh immunoglobulin ("anti-D").

Steroids. Oral corticosteroids have been used for many years for the treatment of ITP in all age groups. They are presumed to act through several mechanisms, including inhibition of reticuloendothelial system phagocytosis of antibody-coated platelets, inhibition of synthesis of antiplatelet antibodies, improved platelet production, and increased microvascular endothelial stability. A variety of dosage regimens have been reported. The traditional regimen is prednisone at 2 mg/kg/day (60 mg maximum) for 14 days with subsequent tapering, discontinuing by day 21. A regimen of 4 mg/kg/day for 7 days, then tapered to day 21 is equally effective. An alternative to these regimens is pulse therapy: dexamethasone 20-40 mg/m^2 (40 mg maximum) for 4 consecutive days, or megadose pulse therapy with methylprednisolone at a dose of 30 mg/kg/day intravenously or orally (1g maximum) for 3 consecutive days. More recently a regimen of prednisone at a dose of 4 mg/kg/day orally for 4 days with no tapering has been shown to be more effective than the conventional standard dose, and may be less toxic than high-dose steroids. Although inexpensive and easy to administer, steroids may be associated with considerable side effects, including weight gain, fluid retention, cushingoid facies, acne, hyperglycemia, hypertension, moodiness, pseudotumor cerebri, cataracts, osteoporosis, avascular necrosis, and immunosuppression with a risk of infection. Toxicity is related to the dose and duration of therapy (5, 9).

Intravenous immunoglobulin. Imbach and colleagues first reported the successful use of intravenous immunoglobulin (IVIG) in the management of acute ITP in a small series of children (38). The effect of IVIG is caused by a competitive inhibition of Fc receptors on phagocytes of the reticuloendothelial system, allowing antibody-coated platelets to circulate. The traditional dose of IVIG is 2 g/kg divided over 2 to 5 days. It has been shown that a single dose of 0.8 g/kg of IVIG has as favorable result as higher doses, and has remained a gold standard for a rapid increase of platelets in children who require treatment. Although more costly than steroids, IVIG is favored by many clinicians as a primary treatment for patients with ITP. Randomized trials have shown that periodic treatment with IVIG increases platelet count more rapidly than treatment with standard doses of prednisone. The yields of platelet recovery, however, are not significantly different from those with higher doses of prednisone. The adverse effects of IVIG are common but

generally mild, occurring in 15 to 75% of patients. They include flu-like symptoms such as headache, nausea, lightheadedness, and fever. Occasionally aseptic meningitis, anaphylaxis, hemolytic anemia, hepatitis C transmission, and hemiplegia are seen (5, 9).

Table 5 Commonly used regimes for treatment of acute ITP in children*

Corticosteroids	Dose
Prednis(ol)one oral	1-2 mg/kg/d for 21 days (maximum dose, 60 mg/d) 4 mg/kg/d for 4 days with abrupt discontinuation (maximum dose, 180 mg/d)
Methylprednis(ol)one oral or i.v.	10-30 mg/kg/d for several days
Dexamethasone oral pulse	20-40 mg/m^2 for 4 consecutive days (every month, 6 cycles)
Immunoglobulins	**Dose**
IVIgG	0.4 g/kg/d for 5 days 1 g/kg/d for 2 days 0.8 g/kg/d once 0.25 to 0.5 g/kg/d for 2 days or once
Anti-D	25 µg/kg/d for 2 days 40-50 µg/kg/d once 75 µg or more /kg/d once

*From: Gadner H. Management of immune thrombocytopenic purpura in children. Rev Clin Exp Hematol. 2001;5:201-221.

Anti-D immunoglobulin. Anti-D is a plasma-derived immunoglobulin prepared from donors with high titers of anti-Rh (D) antibodies. Anti-D can effectively raise the platelet count by blockage of the reticuloendothelial system with antibody-coated red blood cells, thereby minimizing removal of antibody-coated platelets. The patients must be Rh(D)-positive and must have a functioning spleen. Although the optimal dose for anti-D has not been established, the generally recommended dose is 50 to 75 µg/kg as a short intravenous infusion. Responses to anti-D therapy are comparable in magnitude and duration with those observed after IVIG therapy. Anti-D is well-tolerated with only minimal side effects reported in 3% of infusion, including headache, nausea, chills, fever, and dizziness. Hemolytic anemia is the main adverse and inevitable reaction of anti-D, owing to the binding of anti-D antibody to Rh(D)-positive red blood cells. The average decline in hemoglobin concentration ranges from 0.5 to 1 g/dl, and in far the most instances does not require medical intervention.

The most commonly used regimens of steroids, IVIG and anti-D for the initial treatment of acute ITP in children are listed in Table 5. The comparison of these treatment approaches is summarized in Table 6.

Table 6 Comparison of various treatment regimes in children with ITP*

Treatment Response	Prednisone (4 mg/kg/day, d 1–7, max 60 mg)	IV Immunoglobulin (1–2 g/kg)	Anti-D Immunoglobulin (75 µg/kg)
Response > 20.000 at 48 hours	60–70% of patients	70–80% of patients	77% of patients
Common side effects	Weight gain, irritability, hypertension, stomach pain, hyperglycemia	Post-infusion headache, vomiting, allergic reactions, fever, chills	Hemolysis, chills, fever, headache
Rare but severe reactions	Gastric ulcer, reflux, bleeding, hypertension-induced intracranial hemorrhage	Anaphylaxis, aseptic meningitis, renal failure	Massive hemolysis with associated back pain, myalgia, anemia
Duration of initial response (days)	Wide range of response after 30 days of weaning from initial dose to 0	21–72 days with platelet counts greater than 20,000/mm^3	21–48 days based on the 75 µg/kg dose

*From: Nugent DJ. Immune thrombocytopenic purpura of childhood. Hematology Am Soc Hematol Educ Program. 2006:97-103.

On very rare occasions, children with acute ITP and severe thrombocytopenia present with life-threatening bleeding. Management of such cases is challenging and involves measures that have the potential to increase the circulating platelet count rapidly. Appropriate interventions include immediate intravenous administration of methylprednisolone at a dose of 30 mg/kg (maximum dose 1 g) over 30 minutes plus massive (two- to threefold larger than usual) transfusions of donor platelets in an attempt to boost the circulating platelet count temporarily. After administration of methylprednisolone and platelets, an infusion of IVIG at a dose of 1 g/kg should be started, with IVIG and methylprednisolone repeated daily as clinically indicated, usually for 2 or 3 days. Life-threatening hemorrhage is the only indication for platelet transfusion in ITP. The aim of these measures is to maintain platelet counts greater than 50 x 10^9/l. Emergency splenectomy may be considered in individual patients who fail to increase platelet counts or stop bleeding (39, 40). Reports with recombinant factor VIIa are limited but this hemostatic agent should be considered in critical situations (41).

Chronic ITP may present with variable symptoms and platelet counts. In up to 80% of these patients spontaneous remission will occur months or years later. Many children are symptomless and have platelet counts in the range of 20 to 100 x 10^9/l, thus requiring no treatment. Some cases may occasionally need platelet-enhancing therapy, for which the least toxic approach should be chosen. The smallest but most important category is children who have platelet counts less than 20 x 10^9/l and bleeding symptoms (41). In this subgroup second-line therapies or splenectomy may need to be considered. Pharmacotherapy options include short courses or pulses of corticosteroids, or intermittent IVIG or anti-D. The goal of these repetitive treatments is to maintain a hemostatically safe platelet count (5). The novel therapeutic agent is rituximab, a human murine (chimeric) anti-CD20 monoclonal antibody that induces selective depletion of B-lymphocytes necessary for antiplatelet antibody production (42, 43).

Splenectomy should be considered in children with chronic ITP, who fail to respond to drug therapy and have persistent significant bleeding problems and platelet counts usually less than 10 x 10^9/l (44). These conservative guidelines reflect the high rate of remissions that occur in children who have early chronic ITP, and the small but definite risk of overwhelming postsplenectomy sepsis, especially in children under 5 years of age. As the spleen is both a major site of platelet destruction and antiplatelet antibody production, splenectomy may induce sustained remission in the large majority of patients (60 to 90%). When possible, surgery should be performed using laparoscopic techniques. Presplenectomy immunizations and subsequent long-term penicillin prophylaxis is necessary (5, 45).

Children with chronic and severe ITP who fail to remit after splenectomy present very challenging management decisions for pediatric hematologists. No specific therapy recommendations exist for this small group of patients. An array of agents, which have been used primarily in adults, is available (46). They include azathioprine, cyclophosphamide, vinca alkaloids, combination chemotherapy, danazol, cyclosporine A, dapsone, colchicine, and interferon-alpha (47, 48). Thrombopoietin and thrombopoietin-like agents are novel promising thrombopoiesis-stimulating agents (49). The true place of all these approaches in the management of children with chronic refractory ITP remains to be determined through prospective clinical trials.

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Perforation of the terminal ileum secondary to ingestion of duck bone

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Foreign body (FB) ingestion is a common clinical problem. The majority of swallowed object are often passed spontaneously without complications. However, a small proportion of FB that passes from the stomach result in complication distally which require a surgery. We describe an ileal perforation following the ingestion of a duck bone which was diagnosed by CT preoperatively and confirmed by laparoscopy. The case reported is of interest for several reasons, such as the lack of any condition that can predispose patients to accidental ingestion of FB, no specific history of FB ingestion and the small possibility of detecting foreign bodies on computed tomography.

Key Words: Foreign body, Duck bone, Ileal perforation.

Introduction

Perforation peritonitis is the most common surgical emergency noticed in the younger age group. There are numerous causes of gastrointestinal tract perforations, and most of these perforations are emergency conditions of the abdomen that require early surgical treatment (1). Ingestion of foreign bodies is not an uncommon occurrence although fortunately most pass through the gastrointestinal tract without a problem. Foreign bodies such as fish bones (2), chicken bones (3), dentures (4), toothpicks and cocktail sticks (5) have been known to cause gastrointestinal tract perforation especially in certain classes of people. A very small percentage perforates the gastrointestinal tract, leading to acute abdomen and requiring surgical intervention (6). Preoperative diagnosis is usually difficult.

We report a case of perforation of the ileum by a piece of ingested duck bone diagnosed by CT and confirmed by laparoscopy.

Case Report

A 70-year-old previously well man presented to our hospital with a 20-day history of abdominal pain and with a 5-day history of severe abdominal pain with bilious vomit-

ing and fever. On examination, he was febrile, tachypnoeic, and had tachycardia. His pulse rate was 110/minute, blood pressure was 110/60 mmHg. He was irritable with a high fever of 39°C and on physical examination rebound tenderness was present in the right iliac fossa. His white blood cell count was raised at 17×10^9/l. Other laboratory blood and urine tests were normal. Chest radiograph showed free intraperitoneal air under the diaphragm. The original preoperative CT scans were reviewed by a senior radiologist, and it was thought that a small region of localized inflammation containing the possibility of a sharp foreign body, as shown in Figure 1 and an enlarged segment of small bowel and free intraperitoneal fluid were present. He was presumed to have intra-abdominal abscess due to foreign body perforation or appendicitis. On laparoscopy of the case, we found a perforation located at the terminal ileum by a foreign body, 20 cm proximally to the ileo-caecal junction. Extraction of the foreign body could not be achieved laparoscopically. There was also purulent peritoneal fluid on laparoscopic evaluation. Thus, we performed laparotomy (Figure 2). Due to the severe inflammatory changes of the terminal ileum and severe peritonitis, segmental resection of the terminal ileum with ileostomy was performed. The patient had an uneventful postoperative course and was discharged seven days after the procedure.

Discussion

Gastrointestinal perforation is a hole that develops through the entire wall of the gastrointestinal tract and can be caused by a variety of illnesses, such as ulcer disease, appendicitis, less commonly, inflammatory bowel diseases (1). Perforation of the ileum by ingested foreign bodies is uncommon. These foreign bodies may lodge anywhere in the gastrointestinal tract, and less than 1% of ingested foreign bodies perforate the small intestines (2). Abdominal CT scan is considered the most useful imaging to detect foreign bodies or complications arising from them (6).

The mainstay of treatment for gastrointestinal perforation is surgical management. However, if there are no symptoms or signs of generalized peritonitis, nonsurgical management of perforation is a feasible option (7). Treatment usually involves surgery to repair the perforated area. Occasionally, a small part of the intestine must be removed. A temporary colostomy or ileostomy may be needed. The first goal of surgical therapy is to correct the cause of peritonitis. Then the other goals are to correct the underlying anatomical problem and to remove any foreign material in the abdominal cavity. Further, experience and the advancement in accessories have enabled laparoscopic repair of intestinal perforations (8). Marked peritonitis and poor nutritional status were the main factors in this old patient, so preference was given to temporary loop ileostomy.

This case is particularly interesting because peritonitis secondary to perforation due to a foreign body was discovered preoperatively using a CT scan and the definite diagnosis was established by laparoscopy but extraction of the foreign body could not be achieved due to its shape.

Intestinal perforation after foreign-body ingestion must always be considered for the differential diagnosis of acute abdomen even if there is a lack of information about ingestion of a foreign body such as chicken bones preoperatively. Although preoperative diagnosis may be made using an abdominal CT scan, for doubtful conditions, laparoscopy may be more useful, wherefore it allows further examination of the entire abdomen. And as a suggestion, diners should be more careful.

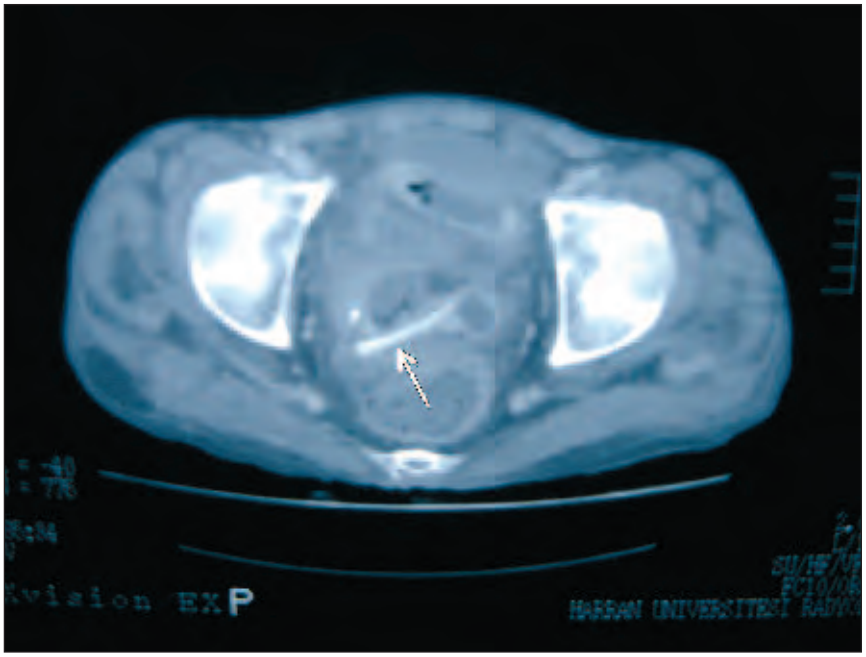

Figure 1 Abdominal CT images of the patient, the arrow is pointing to the foreign body

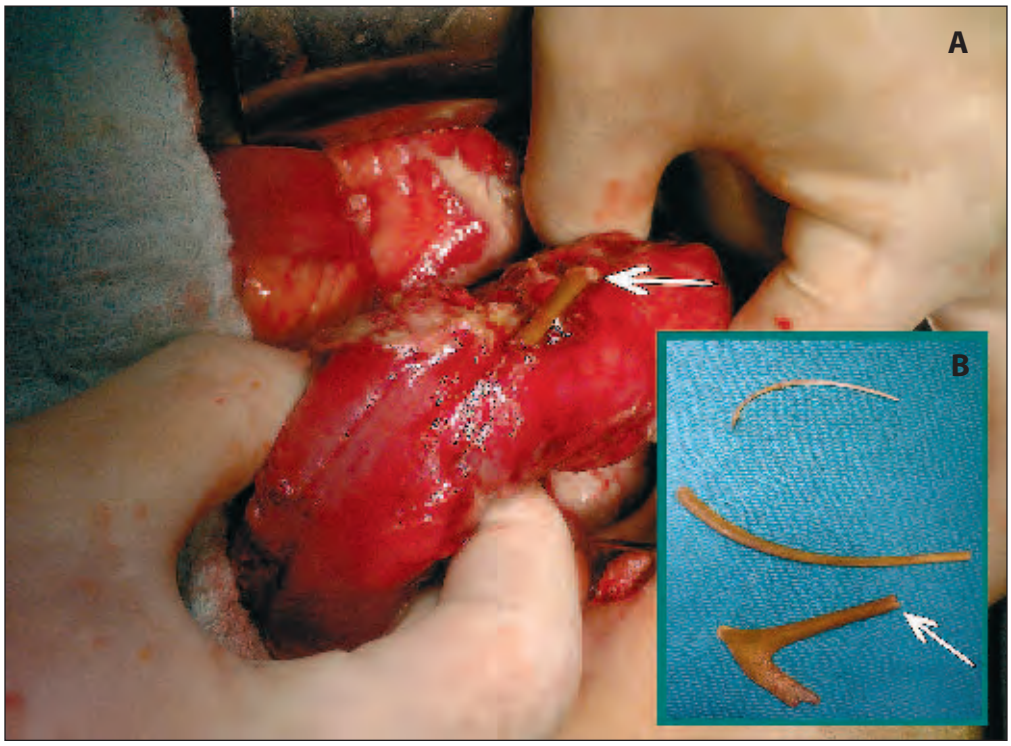

Figure 2 *A* Photograph of the specimen intraoperatively shows a fragment of ingested bone penetrating the ileum. *B* Photograph of the retrieved ingested bones. Both arrows show the same bone and same side

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Objective assessment of diagnostic tests validity: a short review for clinicians and other mortals. Part II

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The whole point of a diagnostic test is to use it to make a diagnosis, thus the obvious need is to know how accurately a particular diagnostic test detects patients with or without a disease. In order to know it, a clinician or a researcher should have a basic understanding of the principles of objective appraisal of diagnostic test. In the second part of this short review, the author presents the most common biostatistical methodology for assessment of a validity of diagnostic tests. Definitions and interpretations of accuracy and likelihood ratio are also provided together with methods of their calculation.

Key words: Accuracy, Likelihood ratio.

Introduction

In the previous part we discussed sensitivity, specificity and positive and negative predictive values. We are continuing with an explanation of accuracy and likelihood ratio. In order to make calculation easier, let us remind ourselves of our imaginary clinical research.

As previously stated, liver biopsy is currently considered to be the gold standard in the assessment of the presence and degree of liver fibrosis in various liver diseases, such as viral hepatitis etc (1). However, it is associated with the possibility of severe complications and serious discomfort for the patient (2). Therefore, our hypothetical investigators decided to evaluate a non-invasive marker of liver fibrosis comparing it against the gold standard (liver biopsy).

Investigators recruited 189 patients. After performing a liver biopsy, 43 of them had liver fibrosis, while 146 did not. On the other hand, after performing a non-invasive test for liver fibrosis, 61 patients were positive for the presence of liver fibrosis, while 128 of them were negative. Now, let us make a 2-by-2 table out of this data (Table 1).

Accuracy

If we want to know the overall accuracy of a test we will need to calculate what proportion of all tests have given the correct result (true positives and true negatives as a pro-

Table 1 Two by two table showing the results of validation study of non-invasive liver fibrosis test against gold standard

	Liver biopsy positive	Liver biopsy negative	Total
Test positive	43 (TP)	18 (FP)	61
Test negative	0 (FN)	128 (TN)	128
Total	43	146	189

TP-true positive; TN-true negative; FP-false positive; FN-false negative

portion of all results) ? (3). For this particular task we will use the formula

Accuracy = (TP+TN)/(TP+TN+FP+FN), or in our case

Accuracy=(43+128)/(43+128+18+0)= 171/189=0,905=90,5%

This means that our test correctly classifies 90.5% of patients (true positives and negatives).

Likelihood ratios

Although the sensitivity and specificity of a test are virtually constant whatever the prevalence of the condition, the positive and negative predictive values depend crucially on prevalence (prevalence in our study=43/189=0,2275=22,75%). When we change the prevalence PPV and NPV change also. The lower the prevalence the more sure we can be that a negative test result indicates the absence of a condition and the less sure we can be that a positive result really indicate the presence of a condition (4).

In order to avoid the impact of prevalence and to express the usefulness of a diagnostic test, likelihood ratios can be calculated. Likelihood ratio (LR) expresses the magnitude by which the probability of a diagnosis in a given patient is modified by the result of a test (5). LR for a test result is the ratio between the chance of observing that result in a patient with the disease in question and the chance of that result in subjects without the disease. The likelihood ratio of a positive test (LR+) answers the question "How much more likely is a positive test to be found in a person with the condition than in a person without it?" (3). It is calculated by using the formula

(LR+)=Sensitivity/(100-Specificity), or in our case

(LR+)=100 /(100-88)=100/12=8.33.

On the other hand, the likelihood ratio of a negative test (LR-) answers the question "How much more likely is a negative test to be found in a person without the condition than in a person with it?" (3) It is calculated by using the formula

(LR-)=(100-Sensitivity)/Specificity, or in case of our hypothetical study

(LR-)=(100-100)/88=0/88=0.

How to use likelihood ratios

Now that we know how to calculate LR, how do we use them? Well, we need the help of another formula:

Post-test odds= Pre-test odds of disease x likelihood ratio

We know that in our imaginary research, the prevalence (or pre-test probability) of liver fibrosis is 22.7%. But, imagine that you are working in a clinical setting where the prevalence of liver fibrosis is higher, say 33%. How useful is the non-invasive liver fibrosis test in this case? First we need to calculate pre-test odds from probability, which is a simple task:

Odds=probability/1-probability=0,33/1-0,33=0,33/0,67=1/2

Now, we calculate the post-test odds for liver fibrosis:

Post-test odds = Pre-test odds of disease x likelihood ratio = 1/2 x 8,33 = 8,33/2 = 4,16/1

Post test odds of liver fibrosis are therefore "4.16 in favor to 1 against". We have the post-test odds, so all we have to do is to convert back to post-test probability using the following formula:

Probability = odds in favor/odds in favor + odds against=
=4,16/4,16+1=4,16/5,16=0,806=80,6%

So, after a patient tested positive with our non-invasive liver fibrosis test in this particular clinical setting, we can conclude that he has an 80.6% chance of actually having liver fibrosis. The rationale for calculating the (LR-) is the same. In order to help in the process of calculating post-test probabilities, we are providing here a nomogram from an original paper by Fagan, for working out post-test probabilities when the pretest probability (prevalence) and likelihood ratio for the test are known (Figure 1) (6).

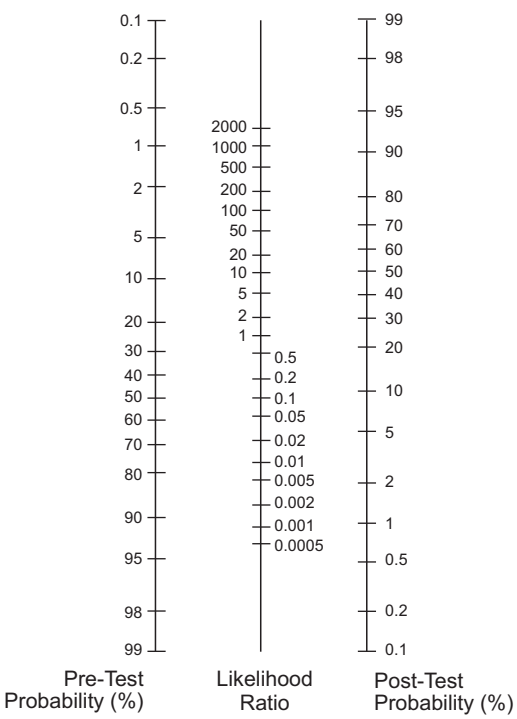

Figure 1 Likelihood ratio nomogram for easy calculation of pre-test and post-test probabilities based on likelihood ratio values

When we have likelihood ratios, the very high or very low prevalence of disease (or pre-test probabilities) are less likely to influence the post-test probability of disease. Although one may question the objectivity of selecting the pre-test probabilities, combining the objectivity of likelihood ratios with subjective pretest probabilities is indeed consistent with the principles of evidence based medicine(7). Although the concept of LR is somewhat hard to grasp, it is of tremendous help in selecting the appropriate diagnostic test based on published results regarding their validity, but in our own clinical setting. They can be used to combine several diagnostic tests; they can be calculated for several threshold values of each particular test (7). An LR greater than 1 gives a post-test probability which is higher than the pre-test probability. An LR less than 1 produces a post-test probability which is lower than the pre-test probability. How much LR actually changes disease likelihood is presented in Table 2.

Table 2 Impact of likelihood ratio values on likelihood of a disease

High LR	Low LR	Impact on Likelihood
>10	<0,1	Large
5-10	0,1-0,2	Moderate
2-5	0,2-0,5	Small
<2	>0,5	Tiny
1	1	No Change

The likelihood ratio thus has enormous practical value, and it is becoming the preferred way of expressing and comparing the usefulness of different tests (3). Moreover, the likelihood concept is applicable in many other situations. Since the likelihood ratio is the ratio of the maximum probability of a result under two different hypotheses, often a null hypothesis and an alternative hypothesis, it can be used as a statistical test (likelihood ratio test) for making a decision

between two hypotheses based on the value of this ratio. It is also possible to use the likelihood concept for calculation of confidence intervals, comparison of two groups, regression models etc, the details of which are well beyond the scope of this article.

We hope that this short review provides the basic information and explanation necessary for a busy clinician to become acquainted with the methodology of diagnostic test assessment. We will continue our series on biostatistics in our next issue with discussion on a different subject.

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