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REVIEW ARTICLE

EXTRACORPOREAL LDL-CHOLESTEROL ELIMINATION IN THE TREATMENT OF SEVERE FAMILIAL HYPERCHOLESTEROLEMIA

Milan Bláha

University Hospital in Hradec Králové: IInd Department of Internal Medicine

Summary: The extracorporeal elimination of LDL-cholesterol could be performed using the classic non-selective centrifuge or membrane plasmapheresis. The modern methods are more selective and effective. The atherogenic particules are removed from plasma by active colon or capsula. The methods include: cascade filtration, imunoadsorbtion, heparin-induced precipitation of LDL, thermofiltration, dextran-induced precipitation of LDL and direct adsorption of lipids (DALI). The regular LDL-apheresis is the life-saving technique in the treatment of homozygous familial hypercholesterolaemia. It is used in heterozygous familial hypercholesterolaemia when the patients do not respond to diet and drugs therapy, too. The regular LDL-apheresis treatment may be followed by the decreased frequency of angina pain episodes, the reduction of ECG changes during the bicycle ergometry and significant disappearance of tendinous xantomas. Some prospective randomised studies has shown even in this group of patients, resistant to conventional treatment, a significant regression of atherosclerotic changes.

Key words: *LDL-cholesterol; Extracorporeal elimination; LDL-apheresis; Familial hypercholesterolemia*

Introduction

Familial hypercholesterolemia and familial combined hyperlipidemia are genetic disorders which are, in their genotype, typical with high incidence of severe cardiovascular complications in young people. When the dietary measures together with combined medicamentous therapy are without any results it is necessary to approach to extracorporeal lipoprotein elimination.

The method of extracorporeal elimination of pathologically accumulated LDL- cholesterol were defined as *LDL-apheresis.*

Importance of LDL-apheresis in familial hypercholesterolaemia could be summarised as follows:

a) Technique is of unambiguous importance in treatment of homozygous patients – it is practically a case of life-saving method. More than twenty years of experience with this method confirmed its unambiguous successfulness. Homozygous familial hypercholesterolaemia occurs in a frequency 1 : 1,000.000 of inhabitants. The cholesterol values rise to 1000 mg/dl (above 20–25 mmol/l). The patients die at an age of 23–25 years due to the complications of premature atherosclerosis. These homozygous patients, representing the highest risk group, allow already to conclude, that regression, primary prevention and secondary prevention can be achieved influencing both the quality of life and prolongation of survival (25). After Bruckert (11) there are three major reasons to treat homozygous patients: 1) their cardiovascular prognosis is very bad 2) drugs are ineffective and surgical procedures such as partial ileal bypass, portocaval shunt and liver transplantation have been associated with considerable morbidity, and 3) LDL apheresis decreases LDL-cholesterol and has been shown to be associated with improved life expectancy (4).

b) It is more difficult to form an opinion on the treatment efficacy in heterozygous patients, as these patients are generally recruited in a more advanced stage of their disease, are older and demonstrate a longer natural survival than homozygous patients. Though regression appears to be possible in young patients, the majority of them experience secondary prevention only, e.g. inhibition of the progress of (coronary) atherosclerosis, accompanied with an increase of the quality of their life. Heterozygous disorders occur in a frequency 1 : 500. The prevailing majority of these patients can be treated with a diet and hypolipidemic drugs only, 5–10 % of these patients have to be treated with LDL-apheresis. It can be concluded in accordance with other authors, that LDL-apheresis seems to be a very useful method in these cases (25).

Methods of extracorporeal elimination of cholesterol

A number of methods for the extracorporeal elimination of cholesterol from intravasal volume can be used

(3,21). These techniques are summarised and shortly characterised in the Tab. 1. We can divide them according to the ways of cholesterol elimination:

– non-specific
– specific
– selective.

Non-specific method means that virtually all other plasma proteins are eliminated together with the substrate to be removed. A selective technique eliminates unrelated plasma substituents together with the pathogenetic substrate, whereas a specific elimination removes the pathogenetic material exclusively. The degree of specificity is often related to the technology applied (3,10).

Plasmapheresis is an example of non-specific and non-selective method. This is an older and well known method of extracorporeal elimination used in haematology (19). The principle consists in removing virtually all plasma proteins together with the LDL cholesterol. It was introduced by De Gennes in Paris in 1967 (12) and later elaborated by Thompson in London in 1977 (15). Blood is taken from one of the peripheral veins, plasma is separated either by centrifugation or by filtration and then removed. It is replaced by substituting fluids, which are crystalloid, colloids and donor's plasma. It was the base of cognition and starting point for application further methods (13,14). The frequent use of plasmapheresis is not desirable (as it is necessary in homozygous patients) with regard to possible side-effects (17).

Cascade filtration: It was described by Agashi et al. (1). Plasma is separated by filtration from other blood components and then perfused through an additional filter. The size of its pores is adapted to separate LDL-cholesterol. This method is suitable especially at the beginning of the therapy because of improvement of the rheological conditions by retaining of fibrinogen, and this way patient's pains caused by angina could be reduced.

HELP – heparin induced extracorporeal LDL- precipitation: This technique was developed by Wieland, Seidel et al. (24). Plasma separated by filtration is exposed to acetate buffer. The insoluble complex of heparin-LDL develops in the presence of heparin at pH 5.12. The excessive heparin is picked up by an adsorber. The complex precipitates in an acid medium and is caught on a polycarbonate filter. Physiological plasma pH is restored by addition of bicarbonate and after volume adjustment, plasma is returned to the patient.

Thermofiltration: This technique was described by Nose (18). Plasma is removed by routine separation, heated to 40 °C and then filtrated through the second membrane. VLDL and LDL cholesterol is separated under thermal conditions, but HDL cholesterol is left. Plasma is returned to the patient after cooling.

Dextran induced LDL-precipitation: This technique was described by Antwiller (2) and Mabuchi et al. (15). In this method the low-molecular dextransulfate linked to micropalets of cellulose is used. The plasmatic lipoproteins containing apoprotein B (LDL, VLDL and triglycerides) are selectively absorbed.

LDL- apheresis:

It is based on immunoadsorption: plasma is pumped through a sepharose gel with coupled sheep antibodies

Tab. 1: The methods of extracorporeal cholesterol elimination (Adapted by Borberg, 1990, 1997).

Author	Method	Advantages/Disadvantages +/-
DE GENNES et al. (12)	Plasmapheresis	+ Rapid elimination - Risk of infection and bleeding - Danger of sensibilisation against administered proteins during a long therapy
AGASHI et al. (1)	Cascade filtration	- Half selectivity, technical reasons, expensive, non accurate separation, lower effectivity, single capsule
STOFFEL et al. (22)	Immunoadsorbtion	+ Selectivity, high effectivity, specificity, no special side effects, flexible, the possibility of regeneration of the used capsules - Technically demanding, expensive
Wieland et al. (12)	HELP (heparin induced LDL precipitation)	+ Half selectivity, no special side effects, effectivity - Technically demanding, difficult, non-specific method, a complicated principle
NOSE et al. (18)	Thermofiltration	+ Selectivity, effectivity - Unclear activity of macromolecules at a higher temperature
ANTWILLER et al. (2)	Dextransulphate induced LDL precipitation	+ Selectivity, high effectivity, no special side effects - Technically demanding
MABUCHI et al. (15)	LDL-adsorption	+ Selectivity, high effectivity, no special side effects, a possibility of capsule's regeneration - Technically demanding
Co. FRESENIUS (9)	DALI (Direct Adsorption of Lipids)	+ Selectivity, no special side effects - Limited capacity, economically demanding

against human apoprotein B 100, which forms the main protein component of LDL-cholesterol.

LDL-apheresis also named immunoadsorption or therapeutic affinity chromatography is the specific extracorporeal on-line LDL-elimination therapy. The concept was suggested by Stoffel in 1981 (22) and introduced into the clinical practice by Borberg also in 1981 (6).

The LDL-apheresis system consists of primary plasma separation generally performed with blood cell separators working at whole blood flow rates from 50 to 80 ml/min and plasma flow rates of 30 to 50 ml/min and a differential plasma separation system consisting of a device automatically performing the repetitive, cyclic loading and desorption of 2 columns (see Fig. 1). The capacity of the system is theoretically infinitive due to 2 different principles. The biochemistry of the adsorber system led with increasing purification and improvement to a loading capacity of 8 g LDL cholesterol per column and even more. Simultaneously the clinical approach is permitting to load and desorb each pair of columns as often as it is necessary for the treatment. The treatment time thus varies according to the pre-treatment cholesterol level of the patient. The essentials of the approach are summarised in Tab. 2 (after Borberg, 1997–6).

As mentioned above, primary plasma separation is generally performed with continuous flow blood cell separators – these devices are well known to the staff in transfusion medicine and have some advantages. The peripheral veno-venous access avoids all potential risks of a central venous or arterial access. As the disposables of blood cell separators are generally less demanding compared to plasma filters, the selection of the appropriate primary plasma separation device is also of economical interest. Last but not least modern blood cell separators are characterised by multipurpose application, are easy to handle – the advantage greatly appreciated by the staff in a blood-transfusion centre (4).

The adsorber columns consists of a sheep antibodies against apoprotein B coupled to sepharose 4B after bromcyanide activation. Beside the advantages of LDL-apheresis due to the specificity of elimination, the capacity of the system and the versatility of the technical device which may be used for all kinds of different adsorbers, the safety of the system was confirmed by many laboratory investigations and statistical data. The LDL-cholesterol can be decreased to the desired normal or subnormal level without a loss of normal plasma proteins or HDL cholesterol (see Graph 1). Long term treatments lead to a steady-state between synthesis rate and removal after 5 to 7 apheresis, if the treatments are performed weekly. The number of days under 200 mg/dl (under 5 mmol/l) is supposed to be crucial for regression of atheromatosis (4,6).

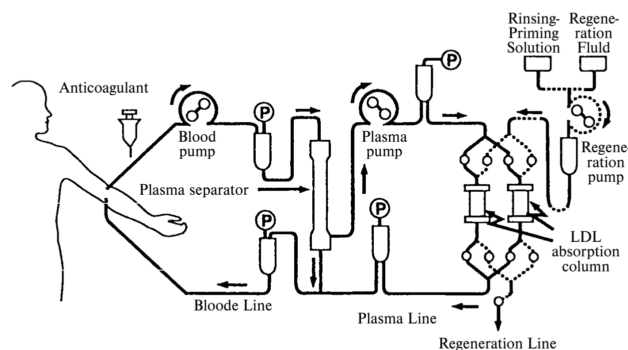
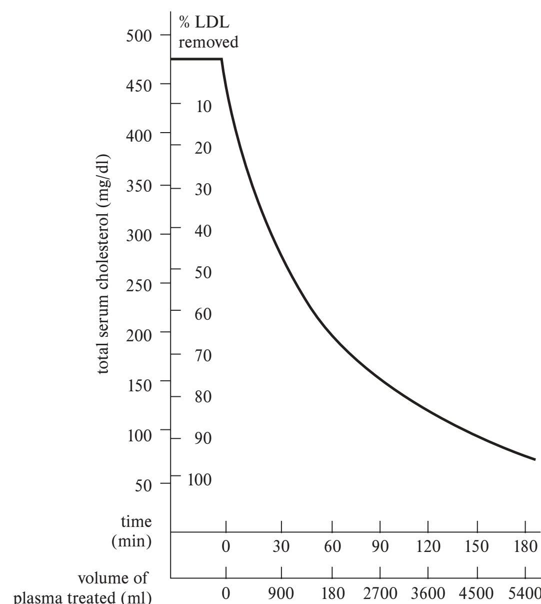


Fig. 1: The principle of LDL-adsorption.



Graph 1: Decrease of cholesterol level during LDL-apherese (after Borberg, 1997).

Tab. 2: Essential of LDL-Apheresis.

I. BIOCHEMICAL	II. TECHNICAL	III. ECONOMICAL
– Therapeutic on-line affinity chromatography	**– Repetive cycling of 2 columns**	**– REUSABILITY (for 50–100 treatments)**
= SPECIFICITY of elimination	**= Virtual indefinite CAPACITY**	
= Reusability	**– Versatility of the secondary separation**	
	= Applicable to all kinds of secondary separation	

DALI – Direct Adsorption of Lipids – the first LDL-adsorption that eliminates LDL cholesterol directly from whole blood, introduced into the clinic by Co. Fresenius, Germany, 1996. This apheresis system is applicable to continuous hemoperfusion of human whole blood. It seems to be very efficient and works without significant side-effects (6,9).

Indications to the therapy

Determination of indication criteria for LDL-apheresis therapy is necessary for these reasons:
- the multidisciplinary approach,
- the social importance due to frequency of severe LDL hypercholesterolaemia in population,
- the relatively high costs connected with secondary atherosclerosis prevention.

The recently achieved degree of scientific knowledge concerning some risk factors for the rise and development of atherosclerosis shows that LDL-cholesterolaemia is the most important risk factor for premature development of atherosclerosis, LDL-cholesterol reduction became the main measure for the primary and secondary prevention of coronary heart disease. Some recommendations for the indication of long-term LDL-apheresis therapy were elaborated in several countries. We are using a modification of these indication criteria (5) – according to the order of importance:

1. Homozygous familial hypercholesterolaemia: a clear and unquestionable indication
2. Patients with hypercholesterolaemia who were unsuccessfully treated by a strict diet and pharmacological therapy, particularly in the following cases:
   a) Coronary atherosclerosis which cannot be resolved cardioasurgically or by methods of invasive cardiology (PTCA, stent) because of disadvantageous anatomical situation or diffuse coronary changes.
   b) Patients after cardiosurgical or invasive cardiological intervention in which high cholesterolaemia survives in spite of the maximal pharmacotherapy and there remains a high risk of the progression of atherosclerotic changes and restenosis in the incriminated region.

Case report

Typical course of the disease and the therapy are described further.

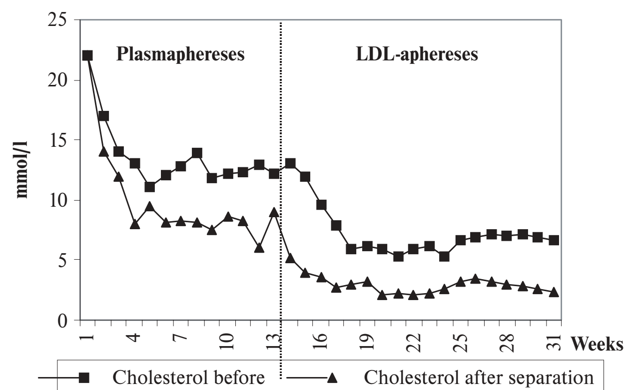
Female patient, born in 1979, was followed at a Centre for nutrition and metabolic disorders, Teaching hospital, Charles University in Hradec Kralove for 12 years.

Serious hypercholesterolaemia and tendinous xanthomas in the area of Achilles tendon and on the back of the palms were found from 6 years of her age. The bicycle ergometry was performed with physiological result and any symptoms of ischaemic heart disease were not proved. Coronarography has not been performed yet. The patient's parents are followed up at the same Centre because of heterozygous form of familial hypercholesterolaemia, their therapy is based on diet and statins, and we have not found any symptoms of heart ischaemic disease yet. The patient's grandmother died of the second myocardial infarct at the age of 68 years, she had her first heart attack at the age of 48 years and according to anamnestical data, she suffered from abdominal angina as well.

Our patient was treated by the sequestrants of bile acids in a dose of 12 g daily since 6 years of her age, she was on a strict diet, but without pronounced effect, though there was a very good compliance given by her family and patient's motivation. The average level of total cholesterol ranged from 15 to 22 mmol/l.

The plasma exchange was the initial method used in the treatment at our department. The cholesterol level at the start is given in the Graph 2. The technique was carried out in weekly interval as far as possible, the cholesterol level decreased, but the effect was not satisfactory – see Graph 2, as the required cholesterol level was not reached. The treatment by LDL- apheresis in a weekly interval was consequently introduced in 1996. (The interval was only exceptionally longer either due to serious personal reasons of the patient or technical reasons.) From 1998, the treatment was carried out in two weeks interval. We washed regularly 7 l of plasma. The cholesterol values after one procedure decreased usually under the level of 2 mmol/l, the mean values were below the base limit, see – Graph 2. It is generally considered as a decisive fact for the prognosis (6,7) and the result was not possible to reach with other accessible methods of treatment. In the first days after the apheresis, the cholesterol level is significantly below the norm. We suppose, that the progress of the disease can be interrupted and resorption of the plaques will continue, because we observe the disappearing of the xanthoms on the hands, their reducing and softening above the Achilles tendon. The time of this treatment is too short to evaluate the therapy in details.

Our patient tolerates the treatment very well, without any detectable side-effects.



Graph 2: The course of treatment – our female patient.

Conclusion

Extracorporeal LDL-cholesterol elimination is the method of choice in a limited group of patients with an extremely high risk of premature or accented coronary atherosclerosis due to a high LDL-cholesterol level. A decision as to whether a particular patient will be treated by some of the method of extracorporeal LDL-cholesterol elimination should be made on the basis of a careful and detailed clinical and laboratory examination of the patient. Our own experience and literary data demonstrate the long-term safety, efficiency and ability of the LDL-apheresis to retard and in most cases to stop or even to cause regression of the coronary atherosclerosis which cannot be resolved otherwise (5,25).

This method is suitable for the primary and secondary prevention of atherosclerosis. LDL-apheresis provides good results from the medical and also from the economic point of view. The number of acute coronary emergencies in high-risk patients (particularly in young people) is reduced and the long-term results of demanding cardio surgical interventions in the coronary bed and the results of PTCA and stent therapy are improved.

Acknowledgement

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Prof. MUDr. Milan Bláha, CSc.,
University Hospital in Hradec Králové,
IInd Department of Internal Medicine,
500 05 Hradec Králové,
Czech Republic.
e-mail: blaha@fnhk.cz

ORIGINAL ARTICLE

PANCREATIC-POLYPEPTIDE IN THE HUMAN PANCREAS: EXPRESSION AND QUANTITATIVE VARIATION DURING DEVELOPMENT AND IN DUCTAL ADENOCARCINOMA

Demetrio Tamiolakis[1], Constantine Simopoulos[2], Athanasia Kotini[3], Ioannis Venizelos[4], Theodoros Jivannakis[5], Nikolaos Papadopoulos[6]

General Hospital of Alexandroupolis: Department of Cytology[1]; Democritus University of Thrace: Department of Experimental Surgery[2], Department of Medical Physics[3], Department of Histology – Embryology[6]; Ippokration Hospital of Salonica: Department of Pathology[4]; General Hospital of Drama: Department of Pathology[5]

Summary: Aim: To determine the immunoreactivity of pancreatic-polypeptide (PP) during the development of the human fetal pancreas and ductal pancreatic adenocarcinoma, given that, PP positive cells were demonstrated either into its embryonic anlage or into pancreatic cancer. Methods: Tissue sections from 15 pancreatic fetal specimens, and equal number of ductal adenocarcinoma specimens, were assessed. Results: The density of positive cells in the primitive exocrine ductal epithelium and endocrine epithelium was significantly higher than the relevant density in the neoplastic pancreatic tissue of mixed (ductal – endocrine) and pure ductal type ($p1=0.001$, $p2<0.0005$, $p3=0.046$ and $p4<0.0005$ respectively). The above values were estimated during the 10th to 12th week. There was no significant difference in the density of positive cells in the mantle zone of the islets from the 13th to the 24th week, and the neoplastic tissue of mixed ($p5=0.11$) and pure ductal type ($p6=0.23$). Conclusion: The immunostaining for PP identifies a subgroup of pancreatic ductal adenocarcinomas with a neuroendocrine component, initially considered as pure ductal tumors, and mixed ductal and neuroendocrine tumors. This pattern of expression in neoplasms recapitulates the normal pattern during the embryonal development of the organ, raising the question of therapeutic efficacy of PP and analogues as potential adjuvant treatment of pancreatic cancer.

***Key words**: Pancreatic-polypeptide; Fetal pancreatic tissue; Pancreatic carcinoma*

Introduction

The development of the endocrine pancreas is complex and interrelated with development of the exocrine portion of the organ. It is now clear that both the exocrine and endocrine pancreas are of endodermal origin (15,16).

The evaginations of pancreatic endoderm (fifth week of gestation) into the investing mesenchyme become tubular structures which branch progressively. The primitive duct epithelium provides the stem cell population for all the secretory cells of the pancreas. It gives rise to α cells which produce glucagon, β cells which produce insulin, and δ cells which produce somatostatin during weeks 8–10. Cells (F-cells) containing pancreatic polypeptide (PP) appear somewhat later. All four different endocrine cell types can be distinguished by immunocytochemistry (8,17). Initially these endocrine cells are located in the duct walls or in buds developing from them.

Pancreatic carcinoma remains one of the most devastating neoplasms of the gastrointestinal tract. Pancreatic cancer is a malignancy that is unresponsive to conventional therapy. More than 85 % of patients have metastatic disease when they are first seen. The incidence of pancreatic cancer is 9 per 100.000 (4) and has remained steady since 1973 (20). Median survival on diagnosis is 11 months, whereas adjuvant treatment (5-fluorouracil and radiation treatment) with surgical resection (Whipple procedure) has extended life by approximately 9 months (11). A dismal prognosis is associated with pancreatic adenocarcinoma despite multimodality treatment protocols. Although total pancreatectomy in selected patients offers survival advantages in rare cases, the difference remains negligible (24). Earlier diagnosis and novel treatment modalities may help to improve survival in patients with pancreatic cancer.

The dismal prognosis of this disease may someday be improved by a better understanding of its pathogenesis. Neoplasms of the pancreas arise from ductal, acinar, stromal, or islet cells. The term carcinoma of the pancreas is customarily used only in reference to exocrine tumors and rare mixed endocrine – exocrine carcinomas. Neoplasms

including carcinomas composed primarily of endocrine cells, are collectively termed islet cell tumors. The precursors of these tumors are presumably developmentally multipotent in terms of their capacity to differentiate into various cell types producing various hormones and regulatory peptides. Whether these cells originate from the ductular epithelium or the islet cells is a matter of debate (13).

Pancreatic polypeptide (PP) was discovered serendipitously nearly three decades ago (12). However, very little is known about its physiologic function or the clinical implications of elevated circulating levels of PP. Human pancreatic polypeptide (hPP) is composed of 36 amino acids. It has been localized within distinct cells in the islet of Langerhans of the pancreas, the F cells that store and secrete PP into the bloodstream (6). Histologically, F cells are abundant in the head and uncinate process of the pancreas (6,22). The release of hPP from the normal pancreas is mediated by the cholinergic nerve fibers that innervate the pancreas. Plasma levels of this linear polypeptide have been shown to increase after a meal, with increasing age, in chronic renal failure, and in patients with islet cell tumors (1,2,14,16).

Previous works describe the growth-inhibiting properties of the peptide YY (PYY), member of the Pancreatic Polypeptide Family, and its synthetic analog PYY(22–36) on human pancreatic ductal adenocarcinomas in vitro (18) and in vivo (19).

PP and its analogues have been included in experimental administration for advanced pancreatic carcinoma patients, based on their antisecretory and antiproliferative properties. However, there has been reported a stimulation effect on neoplastic growth by Ramo et al. (23).

We investigated the immunohistochemical expression of PP in a series of embryonal and neoplastic human pancreatic tissues. We tried to trace the normal expression profile of PP in tissues with different proliferative and differentiating compartments and to investigate whether PP expression in pancreatic carcinoma recapitulates the normal pattern of expression, or may occur as a result of neoplastic deregulation. We conclude that the efficacy of PP administration in pancreatic cancer is yet to be determined.

Materials and methods

Tissue Sampling

The pancreatic tissues were obtained by pancreatoduodenectomy (The Whipple procedure) for carcinoma of the pancreas. Samples from the pancreas of 15 consecutive surgical patients (nine males and six females, aged from 46 to 72 years, average 57.8(11.2) were included in the study. Two tissue samples were taken from each patient: one from the tumor and one from the resection margin. All tumors were verified as pancreatic adenocarcinomas with various degrees of differentiation. The tissues from the resection margins likewise were examined histologically and were found to be free of tumor cells.

Human embryonic (fetal) pancreatic tissue from fifteen fetuses after spontaneous abortion (10 to 12 gestational weeks: 8 samples, 13 to 24 weeks: 7 samples), were investigated.

The local hospital ethics committee approved the use of human tissue, and written informed consent was obtained from all patients.

Immunohistochemical procedure

Pancreatic-Polypeptide immunoreactivity was evaluated using the Lyophilised Polyclonal (NCL-PPp) on formalin-fixed, paraffin-embedded samples. Continuous sections of the tissue were cut into 3–(m thick slices and immunohistochemistry was performed by the avidin-biotin complex (ABC) method, using NOVOCASTRA kits. Briefly, after the sections had been dewaxed and rehydrated, they were washed in phosphate-buffered saline (PBS) and incubated for 30 min in normal goat serum to inhibit nonspecific binding. The sections were then washed in PBS and incubated with antibody against PP (NCL-PPp) overnight at 4 °C. The primary antibody was used after dilution (1:150).

PP (NCL-PPp) immunoreactivity was cytoplasmic, with only occasional and faint nuclear immunostaining. For each sample positive cells in the ducts, islets of Langerhans, aggregates or isolated cells in the pancreatic parenchyme, ware assessed by enumeration of labeled cells in each tissue compartment for a minimum of five random fields per section viewed at 40-fold magnification through a grid. Cell number was calculated per 1 mm^2 of tissue section. The counted areas were selected from random fetal and neoplastic pancreatic tissue sections, taking into account that the ratio of the exocrine pancreatic area (acinoracemose), according to the endocrine pancreatic area (islets of Langerhans) was entirely representative. Statistical analysis was undertaken using the t-test.

Results

Embryonal pancreatic tissue (10 – to 12 – week – old human embryos). During this period of development, endocrine cells (F – cells) demonstrated a strong positive immunoreactivity for PP (NCL-PPp), initially in the primitive exocrine duct epithelium (density of PP positive cells = mean of cells/mm^2 of tissue ± SEM = 37.9 ± 1.6) (Fig. 1) or forming small aggregates (buds) in the surrounding the ductal structures, loose mesenchymal tissue (density of PP positive cells = mean of cells/mm^2 of tissue ± SEM = = 22.3 ± 1.1) (Fig. 2). From the thirteenth to the twenty – fourth week of gestation, period that coincides with the formation of the islets of Langerhans, a strong positive immunostaining for PP (NCL-PPp) was observed to the endocrine cells (F – cells) in the islet cortex epithelium (density of PP positive cells = mean of cells/mm^2 of tissue ± SEM = 27.4 ± 1.3) (Fig. 3).

Neoplastic pancreatic tissue. PP was demonstrated in ten out of fifteen pancreatic adenocarcinomas. The five PP

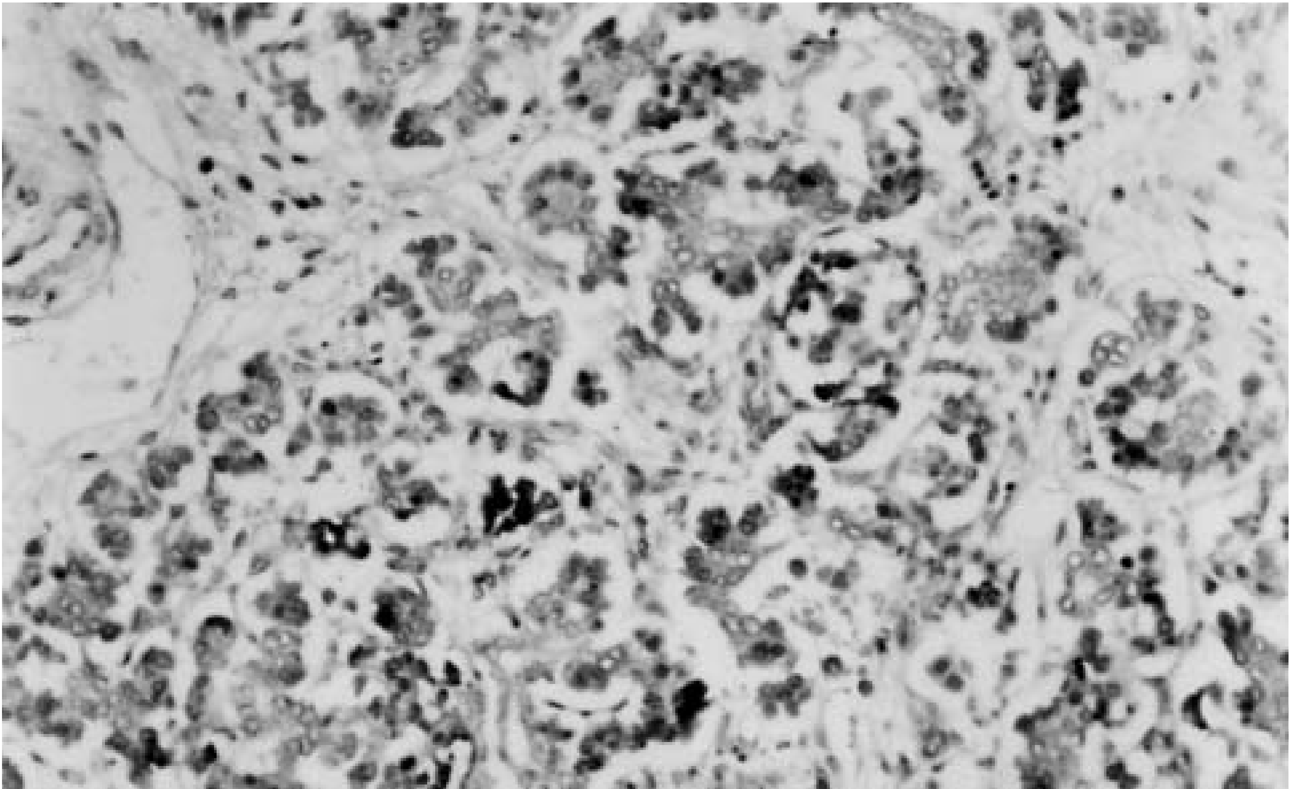

Fig. 1: Pancreatic polypeptide expression in the primitive exocrine ductal epithelium. NCL-PPp X200.

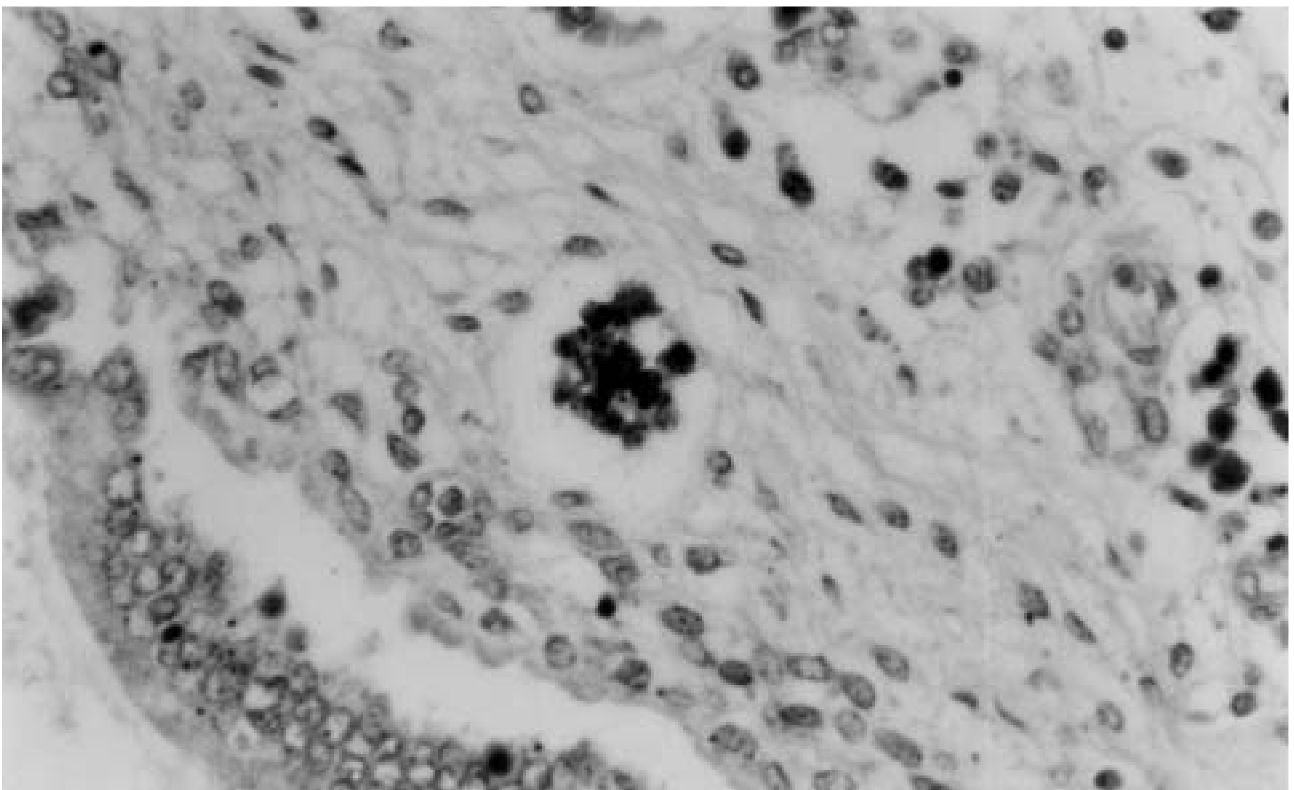

Fig. 2: Pancreatic polypeptide expression in the primitive exocrine ductal buds. NCL-PPp X200.

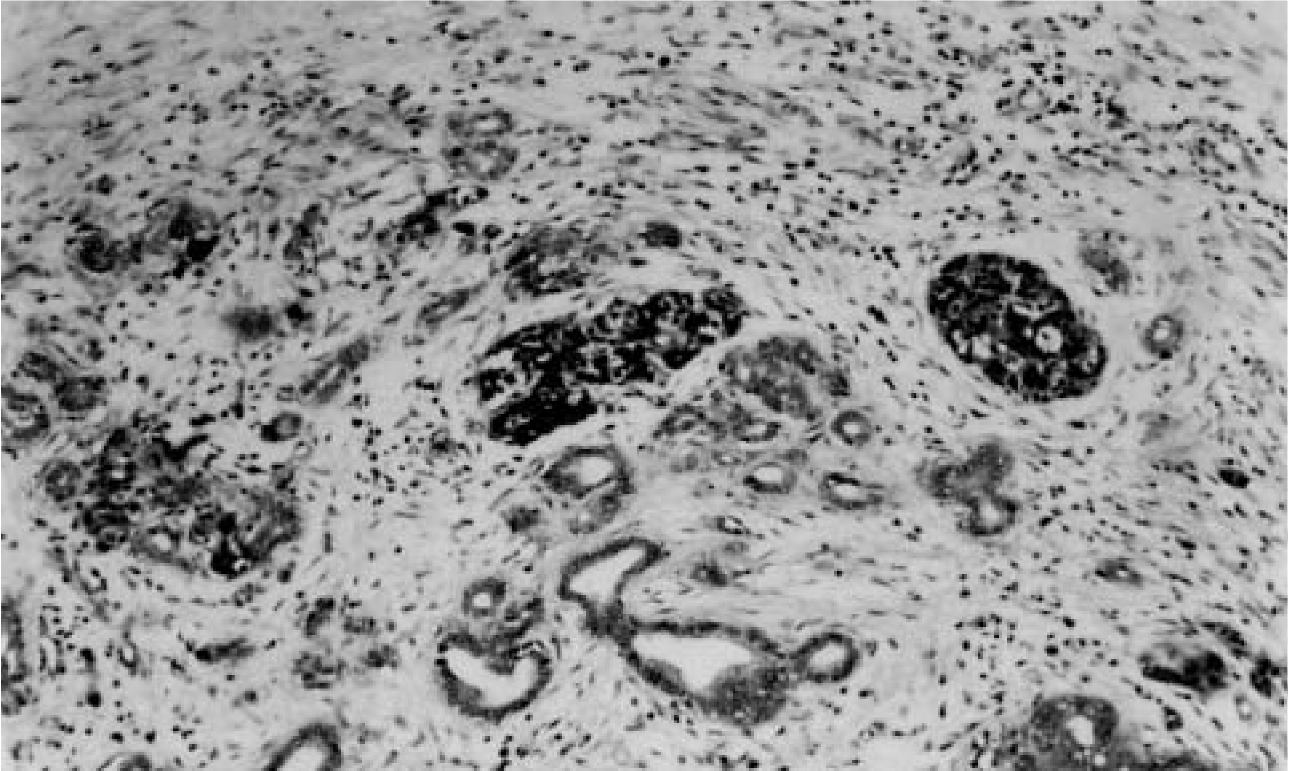

Fig. 3: Pancreatic polypeptide expression in neoplastic pancreatic tissue with recapitulation of the relevant expression of the antigen in the primitive embryonal pancreatic anlage. NCL-PPp X100.

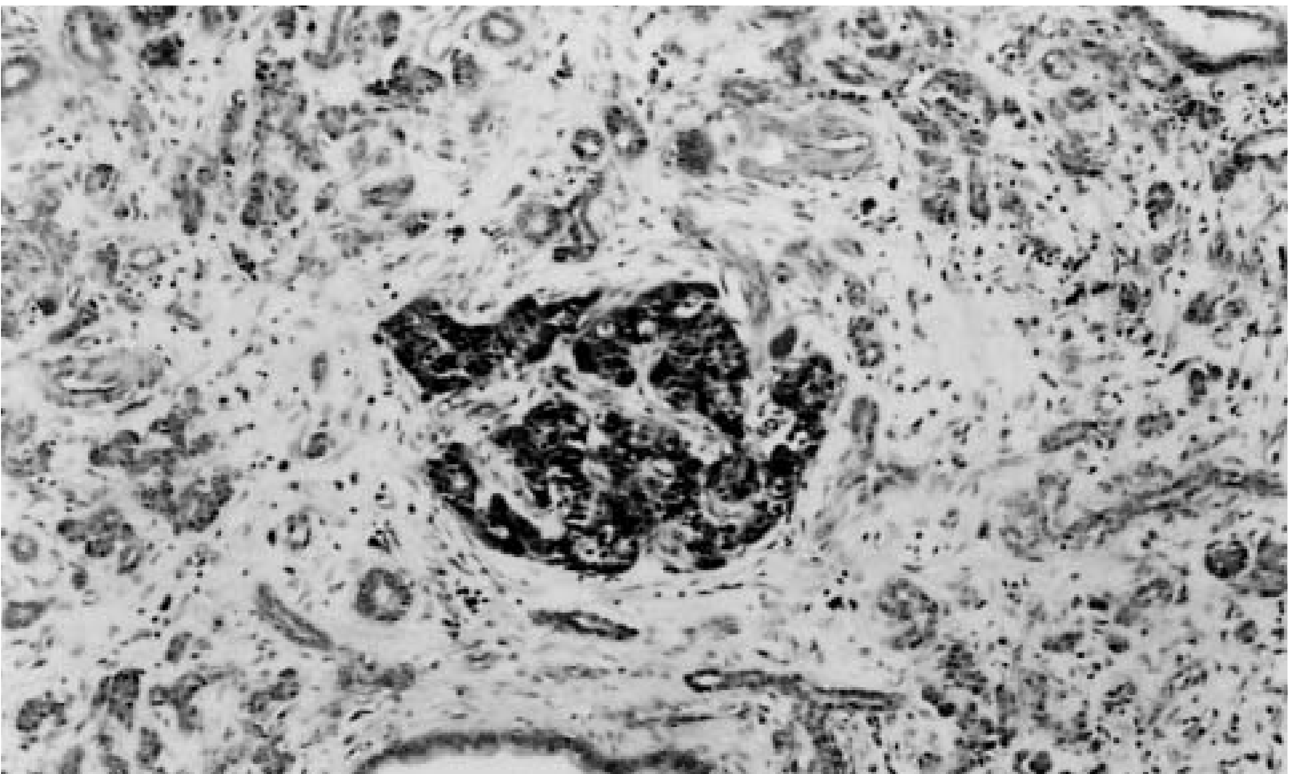

Fig. 4: Pancreatic polypeptide expression in pancreatic adenocarcinoma of pure ductal type NCL-PPp X100.

negative pancreatic adenocarcinomas were of mucinous type. PP positive cells constituted the majority of neoplastic cells in the ductlike structures or small cords of the tumor. Especially, in six cases diagnosed as mixed ductal – endocrine carcinoma, the density of PP positive cells was 29.8 ± 1.2 cells/mm^2 (Fig. 4); in the remaining four cases diagnosed as pure ductal adenocarcinoma the density of PP positive cells was 25.5 ± 1.4 cells/mm^2 (Fig. 5).

There was a statistically significant difference in the expression of PP in the ductlike structures between the primitive exocrine embryonal pancreatic tissue from the 10th to the 12th gestational week, and the neoplastic pancreatic tissue of mixed type (p1=0.001) and pure ductal type (p2<0.0005).

There was also a statistically significant difference in the expression of PP in the buds surrounding the ductal structures between the primitive exocrine embryonal pancreas from the 10th to 12th week, and the neoplastic pancreatic tissue of mixed type (p3=0.046) and pure ductal type (p4<0.0005).

No statistically significant difference was observed in the expression of PP in the islet cortex tissue from the 13th to the 24th week, in comparison with the neoplastic tissue of mixed type (p5=0.11) and pure ductal type (p6=0.23).

Tab. 1: Reactivity of Pancreatic polypeptide (NCL-PPp) in human embryonal and neoplastic pancreatic tissue.

Pancreatic tissue	Number of cases	Density of PP positive cells (average cells/mm^2 of tissue ± SEM)
Embryonal (10–12 weeks)	8	
Primitive exocrine duct walls		37.9 ± 1.6
Primitive exocrine ductal buds		22.3 ± 1.1
Embryonal (13–24 weeks)	7	
Islet cortex epithelium		27.4 ± 1.3
Neoplastic tissue	10	
Mixed ductal-endocrine carcinoma	6	29.8 ± 1.2
Pure ductal carcinoma	4	25.5 ± 1.4

Discussion

The prognosis of patients with exocrine pancreatic cancers remains very poor. Only 36,1 % of patients surgically treated, however, with a 5-year postoperative survival rate of less than 20 % (21). Therefore, new therapeutic approaches for the treatment of exocrine pancreatic cancers must be developed. In the past two decades, the employment of certain gastrointestinal hormones, growth factors, and steroids has been reported in new approaches to control exocrine pancreatic cancers (10).

Pancreatic polypeptide, first isolated by Kimmel and colleagues in 1968, is a 36-amino acid peptide secreted from the F-cells, which are most prominently found in the periphery of the islets in the head of the pancreas (9). Pancreatic polypeptide binds to specific receptors and inhibits exocrine pancreatic secretion of enzyme, bicarbonate, and water and decreases pancreatic blood flow (7). In 1980 Tatemoto and Mutt (25) isolated peptide YY (PYY), and neuropeptide Y (NPY), also 36-amino acid peptides sharing about 50 % homology with pancreatic polypeptide and having similar actions. PYY and its synthetic analog BIM-43004-1 have been shown to cause significant reduction in growth of the human ductal pancreatic cancer cell line MIA PaCa-2 in vitro (18). This analog of PYY was subsequently shown to bind to receptors on these pancreatic cancer cells, decrease intracellular cAMP levels, and suppress tumor growth in vivo (19). By contrast, another study reported increased incorporation of ^{3}H-thymidine in MIA PaCa-2 cells and two other cell lines, one human (Capan-2) and the other a hamster pancreatic adenocarcinoma (H2T), after exposure to NPY and PYY (23). Fisher et al (5) examined the effect of pancreatic polypeptide on the growth of the Capan-2 and H2T cell lines and examined the cells for pancreatic polypeptide receptors using competitive binding assays with ^{125}I-PP. Dose-dependent inhibition of tumor cell proliferation was observed when the H2T cells were cultured with increasing concentrations of pancreatic polypeptide from 10^{-10} to 10^{-7} M. However, no growth effect was detected with Capan-2. Neither cell line could be shown to have pancreatic polypeptide receptors by competitive binding studies. Pancreatic polypeptide, as well as other members of this family of related peptides, may exert an inhibitory effect on pancreatic ductal adenocarcinoma cells. Although Fisher et al were unable to demonstrate receptors in their preliminary unpublished work, others have shown that specific receptors appear to be involved in the mechanism of growth inhibition.

The purpose of our article pointed towards the PP expression in embryonic and neoplastic pancreata. In the fetus, PP was expressed in selected developmental phases suggesting a differentiation – related role. Our data reveal the dynamic behavior of the glandular epithelium in the neoplastic pancreas as well, thus indicating that the human epithelial cells in the branching ducts of the neoplastic pancreas may serve as stem cells, which if appropriately induced may differentiate into endocrine cells such as the F-cells expressing PP. Further studies are warranted to determine the usefulness of this peptide and its analogs, either alone or in combination with chemotherapy and radiation, in the adjuvant treatment of pancreatic cancer.

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Nikolaos Papadopoulos,
Assoc. Professor in Histology-Embryology,
Democritus University of Thrace,
Dragana, 68 100 Alexandroupolis,
Greece.
e-mail: npapad@med.duth.gr

ORIGINAL ARTICLE

EFFECT OF PHOTOREFRACTIVE KERATECTOMY AND LASER IN SITU KERATOMILEUSIS IN HIGH MYOPIA ON logMAR VISUAL ACUITY AND CONTRAST SENSITIVITY

Hana Langrová[1], Matthias Derse[2], Dagmar Hejcmanová[1], Alena Feuermannová[1], Pavel Rozsíval[1], Markéta Hejcmanová[3]

University Hospital in Hradec Králové: Department of Ophtalmology[1]; University Hospital, Tübingen: Department of Ophtalmology[2]; University Hospital, Bohunice, Brno: Department of Ophtalmology[3]

Summary: Purpose: To compare effect of photorefractive keratectomy (PRK) and laser in situ keratomileusis (LASIK) on contrast sensitivity (CS) and best corrected visual acuity (BCVA) in high myopia. Methods: 38 myopes (PRK) and 31 patients (LASIK) were examined before and 1, 3, 6, and 12 months postoperatively. Mean preoperative spherical equivalent was -8.0 ± 1.7D (PRK) and -9.2 ± 2.1D (LASIK). CS was tested on a computerized system of the Contrast Sensitivity 8010 Type at 6 spatial frequencies (0.74 and 29.55 c/deg), BCVA was measured on logMAR charts. Results: At 12 months postoperatively, mean spherical equivalent was -0.6 ± 1.0D (PRK) and -1.0 ± 0.8D (LASIK). Postoperative values of CS were significantly higher in the PRK group, except for spatial frequencies of 3.69 and 7.39 c/deg up to 3 months postoperatively. The initial significant decrease of BCVA lasted up to 6 months after PRK. In the LASIK group BCVA was not significantly different from its preoperative level at the 3-months follow-up. Conclusions: The significant improvement of CS after PRK suggest that PRK can improve quality of vision in eyes with high myopia. Although recovery of BCVA after LASIK was faster than after PRK, there may be a persistent decrease in CS.

Key words: *PRK; LASIK; High myopia; logMAR charts; Contrast sensitivity*

Introduction

Although a number of studies have been published reporting on the results of excimer photorefractive keratectomy (PRK) and laser in situ keratomileusis (LASIK), only few direct comparisons of PRK and LASIK are available. Moreover many studies (1,5,6,17) evaluate the uncorrected visual acuity (UCVA), the best corrected visual acuity (BCVA), the number of Snellen acuity lines gained or lost, and the manifest refraction, which do not enable the detection of subtle changes of postoperative visual functions like reduced night vision and contrast or increased glare. More precise methods, e.g. contrast sensitivity (CS) and glare testing, or examination of threshold on logMAR (logarithm of minimum angle of resolution) charts can provide a better comparison of the advantages and potential risks of both types of refractive surgery. The aim of our study was to compare the quality of vision in patients with myopia above -6.0 D after PRK and LASIK, based on CS and logMAR BCVA testing.

Patients and Methods

Laser treatment was performed using the Multiscan excimer laser system (Schwind, Germany) (3). All patients were treated by two surgeons (P. R., A. F.). Laser parameters included a wavelength of 193 nm, pulse duration of 23 ns, fluence at the corneal plane of 230 mJ/cm^2 and a repetition rate of 13 Hz. The treatment zone diameter was 6.0- or 6.5-mm. For the LASIK procedure, the Mikrokeratom (Schwind, Germany) or the Supratome (Schwind, Germany) were used to prepare a corneal flap of 8.5-mm diameter and 160-μm thickness. Thirty-eight eyes of 38 myopes (23 female, 15 male) with a mean age of 25 years (range 18 to 48 years) were treated by PRK procedure for myopia between -6.0 D and -12.0 D. The astigmatism was up to -4.0 D (mean spherical equivalent -8.0 ± 1.7 D, range -6.0 to -12.6 D). Thirty-one eyes of 31 patients (20 female, 11 male) with a mean age of 24 years (range 19 to 48 years) were treated by the LASIK procedure for myopia ranging between -6.0 D and -13.0 D. The astigmatism was up to -4.0 D (mean spherical equivalent -9.3 ± 2.1 D, range -6.0 to -14.0 D). 20 healthy eyes of 16 women and 4 men (mean age: 26 years, range 20 to 40 years) with no potential relevant eye disease and UCVA of 20/30 or better (Snellen acuity) were examined as a control group. Informed consent was obtained from all subjects. This study was reviewed by an ethic committee. The patients were examined before surgery and at 1, 3, 6 and 12 months postoperatively. The pre-

operative as well as all follow-up visits included a detailed ophthalmologic examination with measurements of BCVA, manifest refraction and CS testing. BCVA was measured under controlled lighting conditions using optotype logMAR charts. The CS was tested using a computerized Contrast Sensitivity 8010 System (Neuroscientific Corp., Farmingdale, USA). The mean CS was calculated and the paired Student's t-test was used for statistical analysis. Differences were considered statistically significant when P-values were less than 0.05. The distance for examination of threshold BCVA on logMAR charts was 4 meters. Each of the 14 rows for visual acuity between 0.1 (20/200) and 2.0 (20/10) contained 10 Landolt rings. Their size in the subsequent rows had a logarithmic progression. The change of about one line on the logMAR charts represented a change in BCVA of about 26 %. It was possible to detect a change of BCVA even of about 1 optotype (10,14). The number of correct answers was noted and the method of Ferris et al. (8) was used for the calculation of the threshold. The distance for CS measurement was 2.2 m so that a range of spatial frequencies from 0.74 to 29.55 c/deg was achieved. The size of the monitor appeared as 5 deg x 3.5 deg in the center of the visual field. CS was measured by the method of ascending and descending limits for six spatial frequencies: two low (0.74; 1.97 c/deg), two intermediate (3.69; 7.39 c/deg) and two high frequencies (14.77 and 29.55 c/deg) (9).

Results

Visual acuity

The preoperative and all postoperative values of BCVA in patients were significantly lower compared to controls (P < 0.001). Preoperative BCVA in PRK group was significantly higher than in LASIK group (P = 0.0002). At the 1-month follow-up BCVA decreased significantly in both groups (P = 0.00007 for the PRK group and P = 0.01 for the LASIK group). The decrease of BCVA lasted up to 6 months

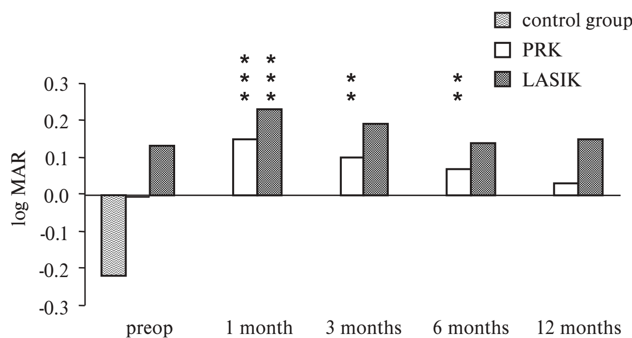

Fig. 1: Mean threshold BCVA (logMAR) of a control group and myopes undergoing PRK and LASIK. Three stars represent a significance at a level of 0.001 and two stars represent a significance at a level of 0.01 between logMAR of myopes after surgery and its preoperative values of the same group.

after PRK but the difference from its preoperative level was not significant at 12 months postoperatively. In contrast in the LASIK group, BCVA improved faster and was not significantly different from its preoperative level at the 3 months and for the entire follow-up. BCVA at the 1- and 3-month follow-ups were not significantly different between both groups, thereafter values of BCVA were significantly higher in PRK group than in the LASIK one (P = 0.04 and 0.0005, resp.) (Fig. 1). At 12 months postoperatively, improvement of BCVA was measured in 51.5 % (PRK) and 51.8 % (LASIK) patients about 1 optotype up to 2.5 lines (equal to 25 optotypes). Four patients of the PRK group lost 2 and 3 lines in BCVA due to significant corneal haze after corrections of -9.25 D to -12.0 D. A detailed evaluation of BCVA changes at 12 months after surgery in lines is documented in Tab. 1 and 2.

Tab. 1: Percentage of eyes with gain of BCVA at 12 months postoperatively in lines.

Lines gained	n	0	1	2	3
PRK	35	8.6	34.3	8.6	0.0
LASIK	31	13.0	32.4	6.4	3.1

Tab. 2: Percentage of eyes with decrease of BCVA at 12 months postoperatively in lines.

Lines gained	n	1	2	3
PRK	35	25.7	11.4	11.4
LASIK	31	29.0	16.1	0.0

Refraction

The mean preoperative manifest spherical equivalent in the PRK group was -8.0 ± 1.7 D and in the LASIK group -9.2 ± 2.1 D. The mean manifest spherical equivalent at 12 months postoperatively were -0.6 ± 1.0 D in the PRK group and -1.0 ± 0.8 D in the LASIK one. At 1 month, a significantly higher number of eyes in the PRK group were within ± 0.5 D of emmetropia compared with the LASIK group. In contrast, at 6 and 12 months postoperatively, a significantly higher number of eyes in the LASIK group were within ± 0.5 D of emmetropia (58.1 % (LASIK) in comparison with 31.4 % (PRK) at 12 months). A residual refraction of ± 1.0 and ± 2.0 D was comparable in both groups at all times (Tab. 3). In both groups, 5.8 % of eyes showed per-

Tab. 3: Percentage of eyes with a manifest refraction of ± 0.5 D; ± 1.0 D and ± 2.0 D at 12 months postoperatively.

time after surgery	surgery	n	±0.5	±1	±2
1 month	PRK	38	73.7	81.6	100.0
	LASIK	28	50.0	71.4	100.0
3 months	PRK	36	50.0	77.8	100.0
	LASIK	28	53.6	75.0	100.0
6 months	PRK	35	45.7	74.3	100.0
	LASIK	29	58.6	75.9	100.0
12 months	PRK	35	31.4	74.3	100.0
	LASIK	31	58.1	80.7	100.0

sistent hyperopia ranging from +0.5 to +1.0 D (mean +0.75 D). A higher percentage of eyes were retreated with PRK (3 retreated eyes; 7.5 %), than with LASIK (no retreated eye). Reoperations were performed for undercorrection between -1.25 D and -3.25 D with no loss of BCVA prior to retreatment.

Contrast sensitivity

The CS of all myopes before and after surgery was significantly lower compared to the controls ($P < 0.05$ to $P < 0.001$) with no significant differences at the lowest spatial frequency in all terms in the PRK group. Preoperatively, there were no significant differences between the PRK and LASIK groups. On the other hand, postoperative data of CS were significantly better in the PRK group ($P < 0.05$ to $P < 0.001$) with the exception of nonsignificant differences at intermediate spatial frequencies at 1 and 3 months postoperatively. In the PRK group, there were no significant changes in CS up to 3 months postoperatively with the exception of a significant increase in CS at the highest frequency ($P = 0.04$ and $P = 0.004$ resp.). Six months after surgery, CS increased significantly at intermediate and high spatial frequencies ($P < 0.05$ to $P < 0.001$). At 12 months after PRK, CS was found to be 99.4 %, 102 %, 105 %, 109 %, 115 %, and 140 % respectively of its preoperative values for the 6 spatial frequencies. On the other hand, in the LASIK group, CS decreased significantly at all spatial frequencies at 1 month postoperatively ($P < 0.05$ to $P < 0.001$). At spatial frequencies of 1.97 and 3.69 c/deg CS remained significantly lower up to 12 months ($P < 0.05$ and $P < 0.01$). At all other spatial frequencies CS increased, but was not significantly lower compared to its preoperative data. 12 months after LASIK, CS reached 96 %, 95.8 %, 96.3 %, 95.8 %, 97.8 %, and 94.6 % respectively of its preoperative values at the 6 spatial frequencies (Fig. 2, Tab. 4 and 5). Typical postoperative changes in CS at spatial frequency of 14.77 c/deg after PRK and LASIK are shown on Fig. 3.

Tab. 4: CS changes after PRK: ↑ represent significant improvement ($P < 0.05$).

Follow up	c/deg 0.74	1.97	3.69	7.39	14.77	29.55
1 month						↑
3 months						↑
6 months			↑	↑	↑	↑
12 months			↑	↑	↑	↑

Tab. 5: CS changes after LASIK: ↓ represent significant decrease ($P < 0.05$).

Follow up	c/deg 0.74	1.97	3.69	7.39	14.77	29.55
1 month	↓	↓	↓	↓		↓
3 months	↓	↓	↓			
6 months			↓			
12 months		↓	↓			

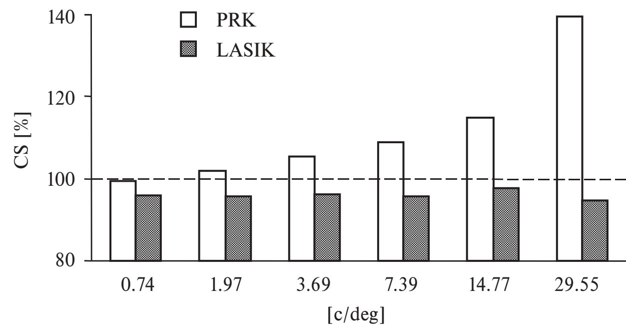

Fig. 2: Contrast sensitivity (%) at 12 months after PRK and LASIK in according to its preoperative values, which represents 100%.

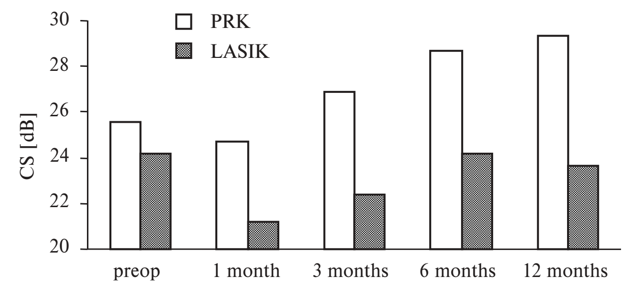

Fig. 3: Typical course of postoperative contrast sensitivity (dB) after PRK and LASIK at the spatial frequency of 14.77 c/deg.

Discussion

In our study, preoperative BCVA was significantly higher in the PRK group compared to the LASIK one, due to the higher myopia in this group. No significant differencies in preoperative CS were found between both groups. Postoperatively, BCVA recovered within 3 months in the LASIK group, whereas in the PRK group there was no significant difference at 6 and 12 months. The decrease of BCVA at 1 month was significantly higher after PRK than LASIK. At the 6- and 12-month follow up, BCVA was significantly higher in PRK group. This is not consistent with finding of Pop et al. (17) who described no statistical differencies in BCVA, refraction and regression for 107 eyes (PRK) and 107 eyes (LASIK) at 6- and 12-months follow ups, even though those groups were greater. The number of over-corrected eyes in both groups (5.8 %) at 12 months postoperatively in our study was higher than that by Pop et al. (17), who reported 3.7 % (PRK) and 1.3 % (LASIK). On the other hand, our higher incidence of reoperations after PRK (7.5 %) than after LASIK (0 %) was lower than those reported by Pop et al. (17) (9.3 % PRK and 2.8 % LASIK reoperations for undercorrection between -1.0 to -2.75 D). Helmy et al. (5) found that regression of the corrective effect was common in both the PRK and LASIK groups, starting between 3 and 6 months after surgery and continuing for up to 1 year. In our study, at the 12-months follow

up, 74.3 % of eyes in the PRK group, compared with 80.7 % of eyes in the LASIK group, were within ± 1.0 D of the desired correction. Only 31.4 % of eyes in the PRK group and 58.1 % in the LASIK group were within ± 0.5 D. These findings are similar to those of Helmy et al. (2). The 6 months results of our study are between the once reported by Hersh et al. (6) (29.4 % of PRK eyes and 27.1 % of LASIK eyes within ± 0.5 D) and the results of Pop et al. (17) (82.0 % of PRK eyes and 71.7 % of LASIK eyes).

Our study showed a decrease in CS at all spatial frequencies up to 3 months in the LASIK group, which corresponded with a decrease of BCVA in the same period of time. Both recovered mainly to the preoperative level. In spite of an even greater decrease of BCVA in the PRK group, CS remained at the preoperative level at 1 month, thereafter CS values increased above the initial level at all spatial frequencies. Postoperative CS was mostly significantly higher in the PRK group than in the LASIK one. The improvement of postoperative CS could be partially caused by a positive learning curve of the patients by repeated examinations as mentioned by Woods and Thompson (20). Pérez-Santonja et al. (15) also described a decrease of CS at low and intermediate spatial frequencies (3 and 6 c/deg) at 1 month after LASIK in 14 eyes with myopia between -6.0 and -19.5 D using the CSV-1000E contrast sensitivity unit (Vector Vision). This drop was followed by a recovery to the preoperative data at 3 months, which is in contrast to our findings. The same authors (15) found a nonsignificant improvement at spatial frequencies of 3, 12 and 18 c/deg at 6 months postoperatively. Nakamura et al. (12) found in myopes with more than -6.0 D a decrease in the 15 % and 2.5 % contrast levels (Contrast-Visual-Acuity-Charts) up to 3 months after LASIK. Mutyala et al. (11) described a significant decrease of CS only at 18 c/deg at 1 week and at 12 c/deg 3 months after LASIK. Holladay et al. (7) found a decrease of contrast threshold in 7 patients, which improved slightly but had not returned to baseline by 6 months after LASIK. The authors (7) supposed that the oblate shape of the cornea following LASIK is the predominant factor in the functional vision decrease. Knorz et al. (8) stated that LASIK seems to cause a reduction of mesopic vision under glare conditions in corrections of more than -5.0 D and in addition that mesopic vision is reduced in myopia over -10.0 D even preoperatively, which is corresponding to our findings. Similar to us Niesen et al. (13) found improvement of CS at 12 months after PRK for spatial frequencies of 12 and 18 c/deg using MCT 6500 in 32 patients with myopia between -2.75 D to -13.63 D. But the majority of authors described only nonsignificant changes of CS after PRK (4,16,18,19) in high myopia.

Further evaluation of larger cohorts is needed to corroborate our findings of visual functions after PRK and LASIK.

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MUDr. Hana Langrová, Ph.D.,
University Hospital in Hradec Králové,
Department of Ophtalmology,
Sokolská 158, 500 05 Hradec Králové,
Czech Republic.
e-mail: langrovah@lfhk.cuni.cz

ORIGINAL ARTICLE

CAN WE PREDICT MAXILLARY SINUS MUCOSA PERFORATION?

Samer Kasabah, Jiří Krug, Antonín Šimůnek, Miguel Cevallos Lecaro

University Hospital in Hradec Králové: Department of Dentistry, Center of Dental Implantology

Summary: This study was carried out to evaluate the prevalence of sinus mucosa perforation occurred during maxillary sinus mucosa elevation surgery, its relation to objective conditions and to the causative medical history, and its influence on postoperative sinusitis, as well. One hundred and forty-six sinus lift procedures have been evaluated in 118 patients. The prevalence of the sinus mucosa perforation was evaluated and subdivided into four groups according to its size and way of treatment. No relation was observed between the perforation and the presence of sinus septa, smoking, radiographic thickening and cyst-like lesions of the maxillary sinus, and previous sinus allergy ($P<0.05$). Despite of high prevalence of the perforation of the mucosa (56.16%), no signs of bone graft infection or maxillary sinusitis were noted in any of our patient.

Key words: *Maxillary sinus; Mucosa perforation; Sinus lift*

Introduction

The aim of the dental implantology is to make implant treatment possible to all patients who may benefit from it. The advanced bone loss and the spongy bone available in the posterior upper jaw pose serious challenges for implant therapy. Recently, the maxillary sinus floor elevation (sinus lift operation) has opened up a new way of placing endosseous implants despite marked bone lack (4,43). One of the most complications during the procedure is sinus mucosa perforation (SMP) (30,37). It is generally agreed that every effort should be made to minimize SMP. However, this is not always possible, because the sinus mucosa is extremely thin, friable, and easily perforated (20). SMP can cause loss of graft material within the sinus that can lead to a sinusitis (3,17,25,26,35,41). Furthermore, a greater bacterial penetration into the graft material through the torn mucosa, and risks of graft contamination can be increased (17). To our knowledge, SMP have not been separately investigated yet.

Materials and methods

All patients who demanded sinus augmentation and endosseous implants were included in this study (118 patients, 58 men and 60 women). The age was ranging from 29 to 58 years with mean of 42 years. Each patient was either partially edentulous in a posterior maxilla quadrant requiring fixed restoration or was totally edentulous indicating fixed removable prosthesis. The medical history including smoking and maxillary pathology-related symptoms was recorded. Patients with a recent history of acute maxillary sinusitis were excluded. Patient was considered a smoker if the patient smokes at least one cigarette daily for more than six months continuously. Preoperative planning consisted of clinical examination of the upper alveolar crest and radiographic assessment with panoramic radiographs. Water's projection or computer tomographies were used when required. This radiographic survey was also used to identify possible maxillary sinus septa. All patients received appropriate antibiotics that were started 24 hours preoperatively and continued for one week.

One hundred and forty-six sinus lift procedures were carried out as described by Boyne and James (4). Once SMP was identified, the sinus elevation procedure was modified and the SMP management was performed depending on the perforation size (Fig. 1, 2). The mucosa was elevated around the perforation, not to enlarge its size and then covered using a small piece of hemostat absorbable fabric (Surgicel®, Ethicon, Johnson&Johnson) to prevent migration of materials into the sinus cavity (Fig. 3). When the SMP was sealed (negative nose-blowing test), the procedure was completed in routine fashion (Fig. 4). We registered the prevalence and the size of SMP. The operated sinuses were comprised into three groups: Group A no perforation occurred during the operation (64 sinuses), in Group B small perforations (< 2 mm diameter) were registered without need of treatment (15 sinuses), and Group C (66 sinuses) included sinuses with all perforation covered by Surgicel®. When SMP was very large and we could not close it, the procedure was abandoned and it could be tried again after a minimum six weeks. It was standard to perform the Valsalv's maneuver in all of our operations to confirm the absence or the sufficient treatment of

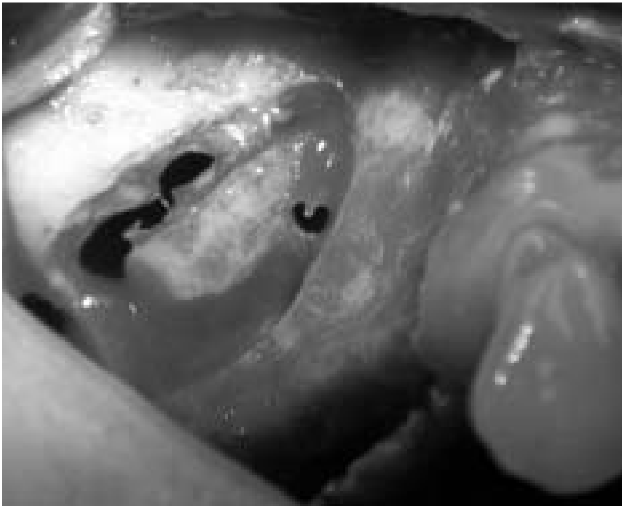

Fig. 1: SMP occurred during the lateral wall osteotomy.

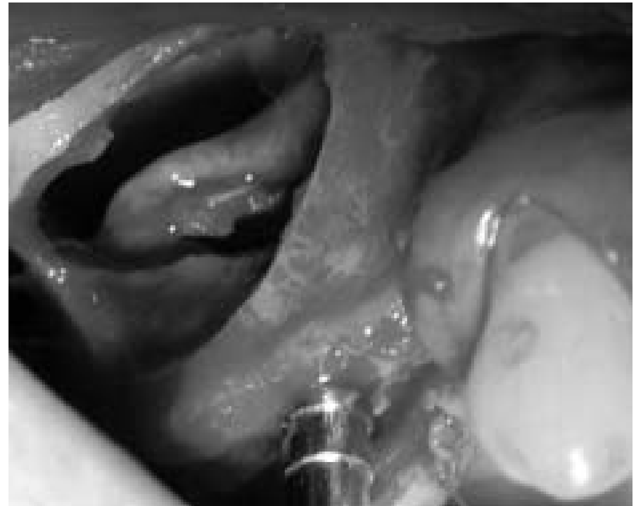

Fig. 2: SMP became larger during mucosa dissection.

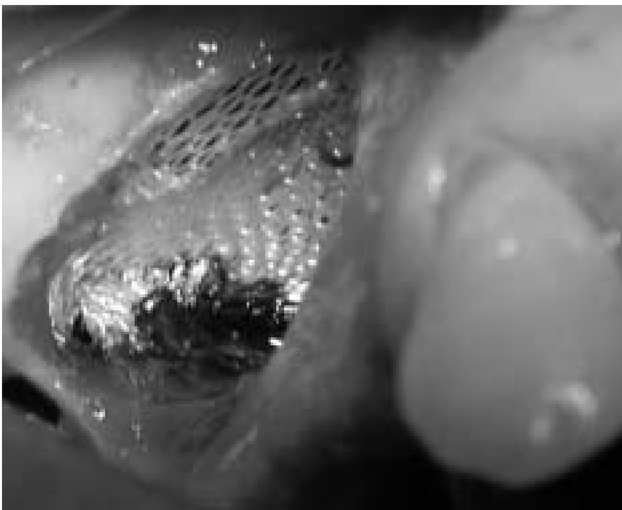

Fig. 3: The mucosa elevation was performed carefully around the SMP and then it was covered with Surgicel®.

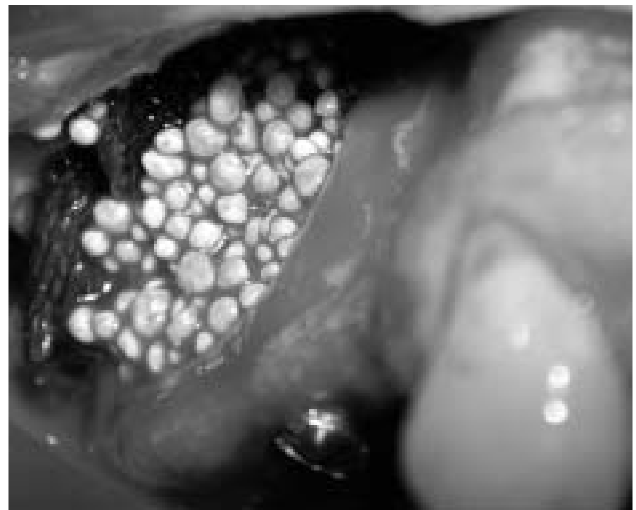

Fig. 4: Once the SMP is sealed, the procedure was completed in routine fashion.

the SMP. We used following augmentation materials: β-tricalcium phosphate ceramic (Cerasorb®, Curasan-Pharma, Kleinostheim, Germany), deproteinized cancellous bovine bone (Bio-Oss®, Geistlich, Wolhusen, Switzerland), and natural red alga (Algipore®, Friadent, Mannheim, Germany). These materials were mixed with the patient's intravenous blood and used alone or in combination with autograft harvested from maxillary tuberosity. Our decision concerning the simultaneous implant placement was not changed if the SMP was properly managed. The patients were called for clinical and radiographic check-ups and asked especially about sinus related pathology (e.g. infection of the maxillary sinus, loss of bone particles through the nose, etc.).

The aim of this study was to evaluate the prevalence of SMP being occurred during the sinus lift procedure, its relation to the objective conditions and causative medical history, and its influence on postoperative sinusitis. The correlation between these conditions and SMP was evaluated statistically using the Fisher's exact test.

Results

Sinus mucosa perforation occurred in 82 of our 146 operations (56.16 %). Table (1) and figure (5) demonstrate SMP size, number, prevalence, and its treatment. The lowest prevalence of SMP was observed in radiographically evident thickening of the sinus mucosa (30 %) (Tab. 2). The prevalence of SMP in radiographically visible cyst-like sinus lesions was 100 % (Tab. 2).

Using Fisher's exact test ($P<0.05$), we did not find any correlations between SMP and all of the above-mentioned findings ($P = 0.101$) and ($P = 0.259$), respectively.

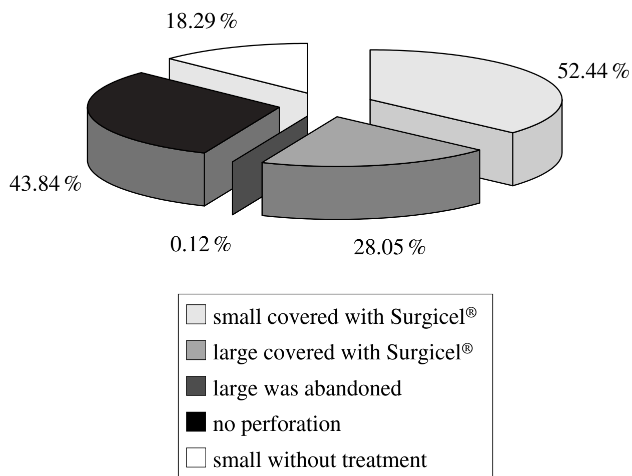

Fig. 5: SMP percentage and its treatment.

Tab. 1: The number and treatment of SMP.

Sinus mucosa perforation (SMP) (n = 82)		
Size	Number	Treatment
Small (< 2 mm)	15	without treatment
Small (< 2 mm)	43	covered with Surgicel®
large (> 2 mm)	23	covered with Surgicel®
large (> 2 mm)	1	abandoned

Some relationship, we figured out in the presence of sinus septa (P = 0.058), but statistically the correlation was not significant.

No relationship was registered in smoking patients (P = 1.000) and in patients with allergic anamnesis (P = 1.000). No relation was found between SMP and smoking patients with sinus septa (P = 0.177).

Despite of high prevalence of SMP, no signs of bone graft infection or maxillary sinusitis were noted in any patient.

Discussion

The present study did not confirm the relation between SMP and the objective conditions as well as the causative medical history related to sinus as was mentioned by some authors (2, 18). We recognized the SMP in 56,16 % of our operations. The prevalence of the SMP in this study was very high in comparison with most other authors. Their results ranged individually from 0 % up to 58 % (0 % Peleg (22), 11 % Leonardis (13), 14 % Loukota, (15), 17 % Wannfors (39), 20 % van den Bergh (37), 30 % Hallman (6), 34 % Timmenga (32), 36 % Raghoebar (26), 40 % Mazor (16), and 16 % to 58 % Krennmair (12). However, these authors did not explain if they published all or only the treated SMP.

It has been mentioned that because the mucosa is strongly adherent to the sinus septa, the elevation of the mucosa without SMP is considered being almost impossible (2,9). In our results, we did not confirm this experience, which can be explained as a consequence of more careful operation technique when the presence of sinus septa was expected. Cigarette smoking is considered as a pathophysiologic parameter that was found to be deleterious to the sinus graft (21,42). We did not notice higher percentage of SMP among smokers. Therefore, smoking can be judged as the pathophysiologic risk factor for the graft success but not for SMP. According to our results, the radiographic thickening of the sinus mucosa, usually indicating chronic sinusitis, allergy etc., did not increase the prevalence of the SMP. Furthermore, it facilitated mucosa elevation. As well as, the radiographic cyst-like lesions of the maxillary sinus did not predispose the patients to a higher SMP percentage. Anyway, it is recommended to treat it before the sinus procedure to avoid graft infection.

Many authors (7,40,41) proved by endoscopic examination the migration of augmentation material particles through the sinus mucosa. From our point of view, there are clinically observed and unobserved SMP. The latter may occur during application of the augmentation materials due to sharp edges of some of them. Many materials have been suggested to cover the perforation (resorbable cellulose membrane, collagen, cortical bone partition, demineralized laminar bone membrane) or it can be glued together with a fibrin sealant (16,27,28,31,36,37). In addition, it is recommended to use a block graft rather than particulated grafts, when perforation is larger than 5 mm (10,31). Pikos (24) described the technique of closure of the SMP using resorbable suture. From our experience Surgicel® is a cheap, satisfactory material for closing of different SMP sizes.

It was surprising that despite of high prevalence of SMP, we registered no sinusitis during our study. Timmenga et al. (32) reported that two sinuses from 85 sinuses developed subacute maxillary sinusitis and in one of these patients the sinus mucosa had been perforated accidentally during the surgical procedure. However, probably some

Tab. 2: Observation results.

	Sinus septa	Cyst-like lesions	Mucosa thickness	Smoking	Allergy	Smoking and septa
The number of the cases	16	3	10	23	6	3
Perforation occurrence	13	3	3	13	3	2
Percentage	81.25 %	100 %	30 %	56.5 %	50 %	66.7 %

transient sinusitis, as Perko (23) mentioned, might happen in a number of our patients, but was not enough obvious to differentiate it from postsurgical symptoms.

There are different ways how to keep the graft mixture in solidified mass and to prevent graft particle migration. Hallman et al (6) added fibrin glue (Tisseel®, Duo Quick Immuno, Vienna, Austria) to graft material to make it easier to handle and to hinder particles from migration in case of SMP. Patient's blood, as we noticed, can act in a similar way as tissue glue in discouraging particle extravasations.

The following rules were essential in all our operations. First, any hole in the mucosa should be closed. When SMP was small, there was no need for further measures because the epithelial lining "falls together" when lifting the door so there is not a great chance of losing graft material into the sinus (37). Second, the sinus mucosa should be completely elevated before graft placement. According to the graft-healing hypothesis in the sinus, mentioned by several authors, the bone formation in an augmented maxillary sinus originates from the floor and the lateral walls and the mucosa does not possess any osteogenetic potential (1,4,7,8,38). That means if there is any unelevated mucosa between the augmentation material and the osseous bed, consolidation will not occur. At the same time the graft will not be secure enough for the placement of osseointegrated implants and predisposes to infection or failure (5). Postoperative maxillary cysts following maxillary sinus elevation were reported. On the histologic examination, their membrane consisted of sclerous sinus mucosa lined with respiratory ciliated epithelium (14,19).

It is known from some authors' observation that less bone formation is depicted close to sinus mucosa (8,18). The sinus mucosa does not have apparent osteogenic potential and its contribution as an angioblast–osteoblast source and/or as endoperiosteum is just becoming understood, but it is probably secondary in importance in sinus graft healing (29). Therefore, the most important task for the mucosa, during sinus grafting, is to act as a net for the graft material.

More than one type of management have been reported how to reduce SMP prevalence. Kent et al. suggested leaving very thin part of sinus wall above the mucosa during osteotomy preparation to avoid SMP with the rotatory instrument. However, the operator must use excessive force to infracture the rest wall of the sinus cavity and this may result in a large tear in the sinus mucosa (11). Ziccardi recommended the use of a diamond bur in contrast to a fluted bur, because it tends to displace the mucosa rather than grab and tear it. Torrella et al. described technique when the sinusal fenestration osteotomy was made by ultrasound technique, which reduced the risk of SMP and improved the quality of the osteotomy (33).

Conclusion

The authors concluded that no relations were noted between SMP and the preoperative condition (sinus septa, smoking, radiographic thickening of the mucosa, radiographic cyst-like lesions of the sinus, and sinus previous allergy). SMP can be problematic but if it is appropriately managed, it does not harm the healing process of the bone graft and could hardly be connected with the development of postoperative sinusitis. On the other hand, it would be convenient to analyze larger number of patients to confirm the relation between SMP and preoperative conditions.

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Samer Kasabah, DDS,
University Hospital in Hradec Králové,
Department of Dentistry,
Center of Dental Implantology,
500 05 Hradec Králové,
Czech Republic.
e-mail: skasabah@hotmail.com

ORIGINAL ARTICLE

HEALTH RISK OF OCCUPATIONAL EXPOSURE IN WELDING PROCESSES I. GENOTOXIC RISK

Lenka Borská[1], Zdeněk Fiala[2], Jindra Šmejkalová[2], Jaroslav Tejral[2]

Charles University in Prague, Faculty of Medicine in Hradec Králové: Institute of Pathological Physiology[1]; Institute of Hygiene and Preventive Medicine[2]

Summary: The welding processes belong among the important sources of occupational pollutions. The welding fumes are ranked, according to the classification of IARC (International Agency for Research on Cancer), into the group of 2B. In our study we have performed the investigation of twenty men (exposed group) working in the stainless steel welding industrial processes (11 welders and 9 grinders, average age was 31 years, 55 % of smokers, average time period of welding occupational exposure was 8 years). The concentrations of chromium (0.557–16.343 mg/m^3) and nickel (0.340–10.129 mg/m^3) in occupational atmosphere highly exceeded established values of maximum permitted concentrations (0.1 and 1.0 mg/m^3, respectively). The concentrations of manganese did not exceed its permitted values. Total concentrations of 12 polycyclic aromatic hydrocarbons (PAH) in occupational atmosphere varied from 300.9 to 961.2 ng/m^3. For purposes of biological monitoring, the levels of chromosomal aberrations were determined in the exposed and control group. Healthy blood donors servedas a control group. People from that group were not occupationally exposed to harmful chemical compounds (20 men, average age was 36 years, 40 % of smokers). Increased level of chromosomal aberrations of exposed group brought the evidence about higher genotoxic risk of investigated welding processes.

Key words: *Welding; Grinding; Occupational exposure; Health risk; Genotoxicity*

Introduction

The technology of welding process belongs among the well-known pollutant sources of occupational environment (7,12,25). Chromium, nickel and manganese belong among the most presented pollutants (26,27,44). The hexavalent chromium and the compounds of nickel are ranked among the proven carcinogens for human (group 1 according to IARC). Metallic nickel is ranked into the group of substances with supposed carcinogenic effect to human (group 2A according to IARC) (18). The degenerative changes of brain structure belong among the most serious toxic effect of manganese (7,10,23,31). Hazardous are also pneumonitis with higher mortality (7,23,31). Tejral et al. (43,44) presented recurring findings of higher air concentration of polycyclic aromatic hydrocarbons (PAH) at welding processes. PAH represent the largest group of chemical carcinogens produced during burning, pyrolysis and pyrosynthesis of organic matter (2,4,5,9,13,14,19).

Luster et al. (30) classify the PAH and the metals mentioned above as the xenobiotics which can cause the immunosuppression and which decrease the resistance of an organism against infection and tumor cells. In addition to described harmful chemical compounds, the ozone and nitrogen oxides are presented at welding processes, too. Noise, vibrations and all kinds of non-ionizing radiation including UV radiation also belong among the risk physical factors at mentioned processes (7,20,23,31). According to IARC, the welding fumes are classified into the group 2B as a possible humoral carcinogens (18). The aim of presented work is focused on the evaluation of genotoxic effects of occupational exposure at welding processes.

Methods

Investigated groups

For our study a group of 20 workers (men), occupationally exposed to welding fumes was chosen (11 welders, 9 grinders, average age was 31 years, 55 % of smokers, average time period of welding occupational exposure was 8 years). The welding of stainless steel materials has been practiced by WIG method in protective atmosphere of argon. All exposed workers filled the questionnaire oriented to their personal history, occupational anamnesis and non-occupational activities. None of investigated workers has been exposed to harmful (genotoxic) chemical substances out of their work.

At the technologies of investigated industrial plant there was no possibility to select an adequate control group of non-exposed workers. Due to this fact the cytogenetic find-

ings of the exposed group of workers were statistically compared with the findings in a group of healthy blood donors (man), marked as a control group (people of various types of occupations, living at the same locality as people from the exposed group). People from the control group were not occupationally exposed to harmful chemical compounds (20 men, average age was 36 years, 40 % of smokers).

Air analysis

The ambient air samples of exposed welders and grinders were taken during their working shift. The air sample collections were executed by personal sampling apparatus SKC (Sampler Aircheck, type PCXR 224, USA) equipped by filters Synpor 4 (diameter 35 mm). Nearly 70 % of working time was covered by the ambient air monitoring. The concentrations of investigated metals (chromium, nickel, manganese) were determined by the atomic absorption spectrophotometry (AAS). The determination was executed according to the hygienic standard laboratory method (17).

The determination of PAH in occupational air was executed by the method of EPA TO-13 (13). Personal sampling apparatus, described above, collected the air samples. The sample analysis was performed by the high performance liquid chromatography (Hawlet Packard 1050) with the fluorescent detection. In each analyzed air sample the concentrations of 12 chosen PAH were determined (phenanthrene, anthracene, fluoranthene, pyrene, benz[a]anthracene, chrysene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, dibenz[a,h]anthracene, benzo[g,h,i]perylene and indeno[1,2,3-cd]pyrene). The total PAH concentrations were calculated as a sum of concentrations of 12 presented PAH.

Chromosomal aberrations

From all persons of exposed and control group the samples of non-coagulated venous blood were taken. The blood samples were analyzed by the standard method of cytogenetic analysis of peripheral lymphocyte (1).

Statistical calculations

For statistic evaluation of our results the "Sigma Stat System" by the Jandel Company (USA) was used. After the control over normality of the data (Kolmogorov-Smirnov test), t-test and non-parametric Mann-Whitney tests were used for the comparison of investigated groups. The statistical process includes the calculation of arithmetic means and standard deviations in particular subsets of analyzed parameters. In the next step, the signification of the differences between calculated means of the subsets was tested.

Results

Air analysis

The concentrations of chromium and nickel in occupational atmosphere of welders and grinders (n = 7) highly exceeded the established values of their maximum permitted concentrations. The concentrations of manganese did not exceed its maximum permitted value (Tab. 1).

Total concentrations of 12 PAH in occupational atmosphere of welders and grinders (n = 2) varied from 300.9 to 961.2 ng/m^3 (Tab. 2). There was no feasibility to compare our results with some permitted values because the maximum permitted concentrations of total (sum) PAH have not been declared. The levels of carcinogenic benzo[a]pyrene were deep below the individual values of its maximum permitted concentrations for occupational environment (11).

Tab. 1: Toxic metals in the occupational atmosphere.

Metals	Range of concentrations (mg/m^3) (n = 7)	NPK-P (mg/m^3)
Chromium	0.557 – 16.343	0.1
Nickel	0.340 – 10.129	1.0
Manganese	0.040 – 1.384	2.0

n = number of analysis
NPK-P = maximum permitted concentrations (11)

Tab. 2: Polycyclic aromatic hydrocarbons (PAH) in the occupational atmosphere.

PAH	Range of concentrations (ng/m^3) (n = 2)
Phenanthrene	278.9 – 900.0
Anthracene	3.7 – 13.1
Fluoranthene	13.5 – 37.2
Pyrene	1.0 – 3.8
Benz[a]anthracene	2.1 – 3.5
Chrysene	1.7 – 2.9
Bezo[b]fluoranthene	1.0 – 1.1
Bezo[k]fluoranthene	<1
Benzo[a]pyrene	<1
Dibenz[a.h]anthracene	<1
Benzo[g.h.i]perylene	<1
Indeno[1.2.3-cd]pyrene	<1
Sum of PAH	300.9 – 961.3

n = number of analysis

Cytogenetic analysis

Twenty people from the exposed group and twenty from the control group were examined for chromosomal aberrations (Tab. 3). In each sample 100 mitotic sets were analyzed. In the exposed group of welders and grinders there were analyzed in a total 2000 cells. From this number, 54 cells (2.70 %) were aberrated. In 12 cases we found the structural aberrations (breaks and exchanges), in 41 cases the polyploidies (numerical aberrations) and one time the

endoreduplication (so called “other type of aberrations”). In the control group there were analyzed 2000 cells. From this number 11 cells (0.55 %) were aberrated. The structural and numerical aberrations predominated. The statistic analysis revealed significantly higher level of aberrated chromosomes of peripheral lymphocytes in the group of welders and grinders.

Reference level of chromosomal aberrations for adults in Czech population (n = 20) has been declared in the range from 0 to 1.88 % (1). Final value 2.70 % of aberrated cells at exposed workers (Tab. 3) was found significantly higher when compared with the reference level ($p<0.001$).

Tab. 3: Chromosomal aberrations of peripheral lymphocytes.

Type of aberration (%)		Exposed group (n = 20)	Control group (n = 20)	p – value	Statistic significance
ABB	Mean	2.70	0.55	0.0000181	***
	SD	1.646	0.973		
SAB	Mean	0.60	0.15	0.03109	*
	SD	0.583	0.654		
NAB	Mean	2.05	0.3	0.0000271	***
	SD	1.532	0.458		
JAB	Mean	0.05	0.05	1	NS
	SD	0.218	0.218		

Mean = arithmetic mean
SD = standard deviation
n = number of analyzed subjects
ABB = aberrated cells
SAB = structurally aberrated cells
NAB = numerically aberrated cells
JAB = aberration of another type
*** = $p< 0.001$
* = $p < 0.05$
NS = non significant

Discussion

Selected toxic metals (chromium, nickel and manganese) occurring in the occupational environment of welding manufactures, dispose of significant toxic potential including the carcinogenic potential (8,15,35,36,41,42).

Beside the welding processes, an excessive exposure to chromium can be found also within manufacturing of alloys and during the galvanic metal coating (16.38). It is known that the toxicity of chromium and chrome compounds increases significantly during inhalation of aerosol particles. Welding fumes represent a typical example of aerosol mixture (18,27,28,29,42,44). According to the scientific data, the welding fumes contain from 18 to 26 % of chromium (mostly in a risk hexavalent form) when the work is executed in so-called inert atmosphere. Other types of working atmosphere contain 3–4 % of chromium (7). For a long time the chromium concentrations in welding fumes were not taken as a health risk factor. Lautner et al. (28) measured the concentrations of chromium particles in the occupational atmosphere of stainless steel welders. The volume of these particles creates 1.9–12.3 % of the total volume of welding fumes. At welding technologies of stainless steel, Edme et al. (12) found an average air level of total chromium 201 $\mu g/m^3$. Karlsen et al. (21) described an average concentration of total chromium 120 $\mu g/m^3$ and an average concentration of hexavalent chromium 21 $\mu g/m^3$.

Many analyses of atmosphere of different workplaces (iron foundries, welding manufactures, battery manufactures) showed that the workers are exposed to nickel in a large range, varying from micrograms to milligrams per cubic meter of air (6,7, 21,22). In the occupational environment of welders, Karlsen et al. (21) determined an average nickel concentration 260 $\mu g/m^3$. Very high short-term exposures to chromium and nickel at welding occupational atmosphere were described in the study of Tejral et al. (43). These exposures highly exceeded the maximum permitted concentrations. They varied from hundreds of micrograms to tens of milligrams per cubic meter for chromium and from hundreds of micrograms to units of milligrams per cubic meter for nickel.

As resulted from our findings, the levels of chromium and nickel occurred highly above the maximum permitted concentrations. The total chromium concentrations varied from 0.557 to 16.343 mg/m^3, nickel concentrations from 0.340 to 10.129 mg/m^3. Maximum permitted concentrations achieve the level of 0.1 mg/m^3 for chromium and 1.0 mg/m^3 for nickel.

The occupational exposure to manganese occurs within metallurgy, electrical, glass making and chemical industry. The coal burning and the metallurgy can be designated as main sources of manganese emissions into the atmosphere (7). In the case of manganese exposure, the respiratory tract is assumed as the most important pathway of exposure. Karlsen et al. (21) found in the breathing zones of welders an average concentration of manganese about 14 $\mu g/m^3$. Tejral et al. (43) presented the findings of very high short-term exposures to manganese in occupational atmosphere of stainless steel welding. These values reached tens milligrams per cubic meter and highly exceeded the maximum permitted concentrations for manganese. All the results of manganese level in presented work were under the maximum permitted concentration (2.0 mg/m^3). They varied from 0.040 to 1.384 mg/m^3.

As it was indicated above, it is necessary to take into account the presence of PAH in occupational environment of welding manufactures (13,32,34,43,44). At presented work, the range of PAH levels (300.9–961.2 ng/m^3) corresponded to our previous findings (43,44). However, it must be noted that presented total PAH concentrations significantly exceeded the findings of other authors from machine-industry environment. For example, concentrations of total PAH which were found in the occupational atmo-

sphere of cutting, dispersion hardening and pressing of the metals varied from 66.9 to 106.0 ng/m^3 (3).

Increased levels of chromosomal aberrations indicate the presence of exposure to some genotoxic factor(s) (37,40). Impaired chromosomes of somatic cells increase the risk of tumors and degenerative diseases in human population. In addition, impaired chromosomes affected negatively the functions of cell repair mechanisms and interfere with the process of apoptosis (37,39,40,45).

The cytogenetic analysis of chromosomal aberrations of peripheral lymphocytes often serves as a biological marker of early genotoxic effects of chemical substances. Knudsen et al. (24) described increased levels of chromosomal aberrations of peripheral lymphocytes in workers exposed to high concentrations of chromium in welding atmosphere. Tejral et al. (43) presented higher levels of chromosomal aberrations in peripheral lymphocytes of stainless steel welders. Myslak et al. (33) confirmed the genotoxic effect of chromium and nickel in welding fumes by the test of sisters' chromatides exchange (SCE) in peripheral lymphocytes. In presented study we described significantly higher occurrence of chromosomal aberrations in the exposed group of welders and grinders. It has to be stressed that the workers were exposed to high concentrations of chromium and nickel from their working atmosphere.

Conclusion

The results of biological monitoring, presented by chromosomal aberrations of peripheral lymphocytes, confirmed higher health risk level for workers exposed to high concentrations of toxic metals and PAH in the atmosphere of welding and grinding processes.

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MUDr. Lenka Borská, Ph.D.,
Charles University in Prague,
Faculty of Medicine in Hradec Králové,
Institute of Pathological Physiology,
Šimkova 870, 500 38 Hradec Králové,
Czech Republic.
e-mail: borka@lfhk.cuni.cz

ORIGINAL ARTICLE

HEALTH RISK OF OCCUPATIONAL EXPOSURE IN WELDING PROCESSES II. IMMUNOLOGICAL EFFECTS

Lenka Borská[1], Zdeněk Fiala[2], Ctirad Andrýs[3], Jan Krejsek[3], Jaroslav Tejral[2]

Charles University in Prague, Faculty of Medicine in Hradec Králové: Institute of Pathological Physiology[1], Institute of Hygiene and Preventive Medicine[2]; University Hospital in Hradec Králové: Institute of Clinical Immunology and Allergology [3]

Summary: Many of epidemiological studies have certified the relationship between welding and various forms of health damages. In our study we performed an immunological research within a group of twenty men, working in the risky environment of manufacturing of stainless steel constructions (11 welders and 9 grinders, average age was 31 years, 55 % of smokers, average time period in welding occupational exposure was 8 years). The exposed group of men was compared with a group of healthy blood donors, marked as the control group (people with various types of employment, living in same locality as a people from exposed group). People within the control group were not occupationally exposed to harmful chemical compounds (from 30 to 100 men were chosen for the individual immune parameters, average age of the whole group was 38 years, 40 % of smokers). When compared with the control group, the exposed group of welders and grinders showed higher level of C3 complement ($p<0.001$), orosomucoid ($p<0.05$), beta-2-microglobulin ($p<0.001$), neopterin ($p<0.001$) and all fagocytic cells ($p<0.001$). On the contrary, in the group of exposed people decreased values of IgA ($p<0.001$), IgG ($p<0.001$), IgM ($p<0.001$), transferin ($p<0.001$), alpha-1-antitrypsin ($p<0.001$), alpha-2-macroglobulin ($p<0.001$), haptoglobulin ($p<0.001$) and ceruloplasmin ($p<0.05$) were found. Some of these changes were characteristic for the exposed group. They could be considered as precursors of biological markers of effect for given type of exposure.

***Key words**: Welding; Grinding; Occupational exposure; Health risk; Immunotoxicity*

Introduction

Apart from the standard forms of biological monitoring of occupational exposure, represented by the analysis of original chemical substances or their metabolites in body fluids and by the analysis of enzyme activity changes, the area of immunotoxicological methods seems to be as a new promising approach (6,21,43). Investigated immune changes can contribute to the elucidation of the way of action of the toxic agents and represent also very sensitive markers of exposure (11,15,21,25,44). The immunotoxicological methods enable us to assess an impairment of the complex immunological processes (anti-infectious, anti-tumour immunity) including an influence of the partial functions of immunity (phagocytosis, production of cytokines, synthesis of antibodies etc.). A substantial effort should be dedicated to the complex description of immune system involving both, immunosuppressive and immunostimulating effects, and leading to the allergic or autoimmune reactions (31,32,46). However, actual scientific literature reported only limited number of immunological findings after occupational exposure (9,11,40,41).

The welding and grinding technologies belong among the well-known pollutant sources of occupational environment (4,5,28,38,39). Many epidemiological studies have already confirmed the correlation between the welding and various forms of health damages, including cancer (3,13,14,16,22,24,36). The malignant diseases were represented as a lung, bladder, throat and pancreatic carcinomas or as an increased risk of myeloid leukemia. In addition to the malignancies, the cases of contact dermatitis (2,33), localized skin erythema and asthma were described (27,34). Higher health risk level of exposure to welding fumes is evident from the facts mentioned above. In spite of this reality, we have found only a limited number of articles, describing the changes of cellular or humoral immunological markers after exposure to welding fumes (6,8,20,41,42). Presented work contributes to the better understanding to the relationships between given exposure and the immunological changes within exposed people.

Methods

Investigated groups

For immunological study a group of 20 workers (men), occupationally exposed to welding fumes was chosen (11 welders, 9 grinders, average age was 31 years, 55 % of smokers,

average time period in welding occupational exposure was 8 years). The welding of stainless steel materials has been practiced by WIG method in protective atmosphere of argon. At the technologies of investigated industrial plant there was no possibility to construct an adequate control group of non-exposed workers. Due to this fact the immunological findings of the exposed group of workers were statistically compared with the findings of a group of healthy blood donors (men), marked as the control group (people of various types of occupations, living at the same locality as people from the exposed group). People from the control group were not occupationally exposed to harmful chemical compounds (from 30 to 100 men were chosen for the individual immune parameters, average age of the whole group was 38 years, 40 % of smokers).

Air analysis

Hygienic characteristics of investigated occupational environment and the results of cytogenetic analysis of exposed and control groups are described in work Borská et al. (7).

Biological samples and immunological methods

Coagulated and non-coagulated blood samples were collected from all persons of exposed and control groups. Phagocytic activity was tested through the yeast's ingestion and expressed as the count of phagocytic and phagocytosis capable cells. ELISA method was used to determine serum level of neopterin and beta-2-microglobulin. ELISA method was also used for the determination of IL-1 beta (Quantikine RDS, USA) and the total IgE (Immunotech, France) concentrations in serum. The IgE, IgA and IgM levels, C3 and C4 components of the complement, alpha-1-antitrypsin, alpha-2-macroglobulin, ceruloplasmin, orosomucoid, prealbumin, haptoglobin, transferin and CRP were determined by nephelometry methods, through the immunoreagencies of Beckman Company (USA).

Statistical analysis

For statistic evaluation of our results the "Sigma Stat System" by the Jandel Company (USA) was used. After control over normality of the data (Kolmogorov-Smirnov test), t-test and non-parametric Mann-Whitney tests were used for the comparison of investigated groups. The statistical process included the calculation of arithmetic means and standard deviations in particular subsets of analyzed parameters. In the next step, significance of the differences between calculated means of the subsets was tested.

Results

Cellular immunity

When compared with the control group (47.5 ± 8.25%) the exposed group of welders and grinders showed increased count of all phagocytic cells (61.15 ± 8.21%) on the highest level of significance ($p<0.001$) (Tab. 1).

Tab. 1: Statistical comparison of the exposed group of welders and grinders with the control group.

parameter	Mean ± SD (welders)	n (welders)	Mean ± SD (controls)	n (controls)	p value	test
Phag. efficiency of leuco (%)	61.15 ± 8.21	20	47.5 ± 8.25	50	< 0.001	t-test
Phag. efficiency of phago (%)	50.5 ± 10.9	20	NO	NO	NO	NO
IgG (g/l)	9.46 ± 1.01	20	13.39 ± 3.06	100	< 0.001	Mann-Wh
IgA (g/l)	2.08 ± 0.67	20	2.75 ± 0.77	100	< 0.001	Mann-Wh
IgM (g/l)	1.10 ± 0.33	20	1.70 ± 0.72	100	< 0.001	Mann-Wh
C3 complement (g/l)	1.05 ± 0.19	20	0.71 ± 0.19	100	< 0.001	Mann-Wh
C4 complement (g/l)	0.29 ± 0.07	20	0.30 ± 0.09	100	0.4	Mann-Wh
IgE total (IU/ml)	269.55 ± 405.81	20	129.30 ± 207.49	100	0.458	Mann-Wh
Beta-2-microglobulin (mg/l)	1.44 ± 0.16	20	0.92 ± 0.38	48	< 0.001	Mann-Wh
Neopterin (nmol/l)	11.86 ± 2.08	20	8.35 ± 2.62	48	< 0.001	t-test
IL-1beta (pg/ml)	3.35 ± 1.34	20	2.59 ± 1.45	35	0.062	t-test
Transferrin (g/l)	2.52 ± 0.32	20	3.00 ± 0.45	50	< 0.001	t-test
Alpha-1-antitrypsin (g/l)	1.45 ± 0.23	20	3.15 ± 0.68	50	< 0.001	t-test
Alpha-2-macroglobulin (g/l)	1.54 ± 0.28	20	2.05 ± 0.52	50	< 0.001	t-test
Haptoglobin (g/l)	1.25 ± 0.53	20	1.62 ± 0.31	50	< 0.001	t-test
Orosomucoid (g/l)	0.89 ± 0.18	20	0.75 ± 0.18	50	0.004	t-test
Prealbumin (g/l)	0.31 ± 0.03	20	0.32 ± 0.05	50	0.943	t-test
Ceruloplasmin (g/l)	0.37 ± 0.05	20	0.42 ± 0.08	50	0.018	t-test
CRP (g/l)	2.93 ± 6.27	20	2.50 ± 1.25	50	0.642	t-test

Mean – arithmetic mean; SD – standard deviation; n – number of persons; NO – no data; Mann-Wh – Mann Whitney test

Humoral immunity

The parameters of humoral immunity of the exposed group revealed a number of differences in comparison with the control group (Tab. 1). Significant elevation ($p<0.001$) has been observed within the levels of C3 component of the complement (exposed group 1.05 ± 0.19 g/l; control group 0.71 ± 0.19 g/l), neopterin (exposed group 11.86 ± 2.08 nmol/l; control group 8.35 ± 2.62 nmol/l) and beta-2-microglobulin (exposed group 1.44 ± 0.16 mg/l; control group 0.92 ± 0.38 mg/l). The increase on the level of significance of $p<0.01$ has been observed in the case of orosomucoid (exposed group 0.89 ± 0.18 g/l; control group 0.75 ± 0.18 g/l).

On the contrary, significant decrease ($p<0.001$) has been found within the levels of immunoglobulin IgA (exposed group 2.08 ± 0.67 mg/l; control group 2.75 ± 0.77 mg/l), immunoglobulin IgM (exposed group 1.10 ± 0.33 g/l; control group 1.70 ± 0.72 g/l) and total immunoglobulin IgG (exposed group 9.46 ± 1.01 g/l; control group 13.39 ± 3.06 g/l), transferin (exposed group 2.52 ± 0.32 g/l; control group 3.00 ± 0.45 g/l), alpha-1-antitrypsin (exposed group 1.45 ± 0.23 g/l; control group 3.15 ± 0.68 g/l), alpha–2–macroglobulin (exposed group 1.54 ± 0.28 g/l; control group 2.05 ± 0.52 g/l) and haptoglobin (exposed group 1.25 ± 0.53 g/l; control group 1.62 ± 0.31 g/l). The decrease on the level of significance of $p<0.05$ has been observed in the case of ceruloplasmin (exposed group 0.37 ± 0.05 g/l, control group 0.42 ± 0.08 g/l).

Discussion

Heavy metals (4,10,23,29) belong to the dominant harmful inorganic agents generated by the welding processes. In described occupational environment it is also possible to find even a lot of hazardous organic materials (for example polycyclic aromatic hydrocarbons) and physical agents (UV radiation) (17,18). According to the great variability of welding environment, it was quite difficult to perform an efficient group of tests for immunological evaluation (monitoring) of occupational risk (6).

The immune system takes part on keeping the homeostasis under variable conditions of internal and external environment (37). It keeps this role due to the ability of macrophages to swallow up noxious agents, too. This process – phagocytosis – is a significant mechanism of body defense and represents the most important part of natural nonadaptive immunity (11). It is also important factor of inflammation and autoimmune reactions. The number and activity of phagocyting cells that are in a direct contact with noxious agents can indicate the load of immune system. Scientific data about the influence of welding operations to phagocytosis are numerous but the results are often controversial in the meaning of the immunosuppressive and immunostimulant effects (20). In our previous study we have found a significant decrease in the total number of all phagocytic cells in the group of welders, working in an average 15 years with the stainless steel (6). In presented study we have found a significant increase of the total number of all phagocytic cells in the group of welders and grinders (workers were exposed to the welding fumes in an average about 8 years). According to these facts we suppose the occupational air contamination and exposure duration as the most important factors of phagocytosis influence.

Neopterin is produced by macrophages. The production is under influence of interferon gamma. Neopterin seems to be a sensitive indicator of immune activation (particularly the cellular activation) (45). It can also serve as a sensitive marker of changes in the system of cytokines that enable to evaluate the endogenous activity of cytokines. It has been proved that the majority of diseases, associated with activation of immune system (including high secretion of neopterin in all malignant tumors), increase the level of neopterine in urine and plasma (19,35). In accordance with the facts described above and with the results of our previous study (6) we have found significantly higher level of neopterine in exposed group of welders and grinders, too.

Beta-2-microglobulin is a part of HLA-I complex situated on a cell membranes. The increase of beta-2-microglobulin activity, as an important marker of activation of immune system, can be observed within many immunopathological and infectious conditions (26,30). Increased expression of beta-2-microglobulin is associated with higher exposure to antigenic substances. This situation indicates the presence of an exogenous substance in organism. The expression is usually being influenced by many factors, among others even by interferones and tumor necrosis factor alpha (12). In our study we have found significantly higher level of beta-2-microglobulin in exposed group of welders and grinders. In our previous study we have found significantly higher level of beta-2-microglobulin only in the case of exposed welders (6).

Pluripotent cytokine IL-1-beta represents key cytokine, produced by the antigen presenting cells (1). The changes within its concentration can be expected anywhere, where the immune system encounters an exogenous material (12). In a contrast to the results of our previous study (6) we have not found significantly increased level of IL-1-beta in the exposed group of welders and grinders.

The complement belongs among the most important defense mechanisms of an organism. Recognition of the antigen by specific antibody starts its classical cascade activation (12). For description of the complement function, the determination of its two important components C3 and C4 was chosen within presented study. Significant increase in the C3 component level in exposed welders was described by Hanovcová et al. (20) while significant increase in the C4 component level was described by Ulrich et al. (42). When we compared exposed group with the control we have found significantly higher level of C3 component only.

Production of antibodies is indicated as a principal function of specific humoral immunity (12). About 10 % of total plasma immunoglobulins belong to IgM class. The

IgM class is an antibody of early immune response for the most of antigens and represents also the most effective immunoglobulin class in the system of the complement fixation. All authors, Ulrich et al. (42), Hanovcová et al. (20) and Borská et al. (6) mentioned significant immunosuppressive effect of welding processes on the IgM level. We observed important decrease of the IgM level in stainless steel welders and grinders even in presented study.

About 75 % of total plasma immunoglobulins belong to IgG class. Boshnaková et al. (8) and Hanovcová et al. (20) have already observed decreased levels of IgG immunoglobulins in welders. Significantly lower IgG levels in exposed welders and grinders have been found also in presented study.

About 15 % of total plasma immunoglobulins belong to IgA class that is presented in both, monomeric and polymeric form. Wagner et al. (44) described significantly higher IgA levels in grinders. On the contrary with their results we found a significantly lower IgA levels in the exposed group of welders and grinders.

The interactions of chemical substances with endogenous macromolecules can lead to hypersensitive reactions. The simplest marker of the 1st type of hypersensitivity is a total level of IgE immunoglobulins. This indicator is very often markedly increased within the people suffering from atopy (12). In our previous work (6) we found significantly higher levels of total IgE in welders, working in an average about 15 years in welding processes. In presented study has not been found any significant difference in investigated levels of total IgE between exposed group (working in an average about 8 years in welding processes) and control group. We suppose that duration of professional exposure can play a significant role in variation of IgG expression, similarly as in the case of variation of phagocytosis.

The levels of proteins which are called "acute phase proteins" (alpha-1-antitrypsin, alpha-2-macroglobulin, transferin, orosomucoid, ceruloplasmin, haptoglobin, prealbumin and C-reactive protein) varied markedly during enhanced load of organism (trauma, surgery operations, malignity, stress, infections). Some of these proteins perform special transport functions and their levels are decreased during the acute inflammation (transferin, haptoglobin, prealbumin). The levels of other proteins are usually elevated during inflammation (CRP, alpha-1-antitrypsin, alpha-2-macroglobulin, orosomucoid, ceruloplasmin) (12). In accordance to our previous results (6) and to the results of Hanovcová et al. (20) we found significant changes of the proteins of acute phase when exposed and control groups were compared.

Conclusion

The complex character of exposure of stainless steel welders and grinders exclude simple applications of immunological tests for purposes of biological monitoring. However, in a large spectrum of used immune markers we have found a number of significant variations particularly characteristic for observed exposed group. We could think about these changes like about the precursors of biological markers of effect in early phases of damage of an organism.

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MUDr. Lenka Borská, Ph.D.,
Charles University in Prague,
Faculty of Medicine in Hradec Králové,
Institute of Pathological Physiology,
Šimkova 870, 500 38 Hradec Králové,
Czech Republic.
e-mail: borka@lfhk.cuni.cz

CHEMOTHERAPY IN A PATIENT WITH PRIOR HISTORY OF IDIOPATHIC THROMBOCYTOPENIC PURPURA

Kouji Kanemoto, Hiroaki Satoh, Kiyohisa Sekizawa

University of Tsukuba: Division of Respiratory Medicine, Institute of Clinical Medicine

Summary: We described a 67 years old small-cell lung cancer patient with a prior history of idiopathic thrombocytopenic purpura (ITP) who was treated with successful chemotherapy. It is probably safe to administer chemotherapeutic agents for some cancer patients with prior history of ITP, but it is important to prevent or minimize the toxicities of these chemotherapeutic agents.

Key words: *Idiopathic thrombocytopenic purpura*

Introduction

The association of idiopathic thrombocytopenic purpura (ITP) with malignant diseases of the lymphoid system is common (1,2,4,5), however, non lymphoid malignancies associated with ITP have also been observed; lung cancer has been one of the most common solid tumors reported (3,7,9,10). We show a lung cancer patient with prior history of ITP who was treated with successful chemotherapy.

Case report

A 67-year-old man was admitted because of productive cough. The patient had a prior history of ITP 6 years previously. The chest x-ray revealed a mass in the left lung with mediastinal lymph node adenopathy. Performance status (ECOG) (11), of the patient was 1. on admission, the platelet associated IgG was increased (62.7 ng/107 cells) (normal range: 0-25 ng/107 cells), while thrombocyte count was 274 x 109/L. Transbronchial biopsy of the tumor was performed, and histologic evaluation revealed a diagnosis of small cell lung cancer (SCLC). Examination by systemic survey revealed no metastases. The patient was informed about the diagnosis and the risk associated with chemotherapy. However, the patient agreed to receive chemotherapy, then he underwent two courses of chemotherapy with carboplatin (area under the curve: 5 mg/ml per minute, Calvert formula (8), day 1) and etoposide (100 mg/m^2, days 1, 2, and 3) underwent. The response to the therapy was evaluated as a good partial response. Thereafter the patient received 2 other courses of the chemotherapy with thoracic irradiation (70 Gy) concurrently. In each course of the chemotherapy, the patient did not develop grade 3-4 myelosuppression (NCI toxicity criteria). Two months after the end of the chemoradiotherapy, the patient developed lumbar bone metastasis, which was treated with irradiation successfully. He is still alive 12 months from the beginning of the chemotherapy without any episode of ITP, although the elevated level of platelet associated IgG continues (62.8 ng/107 cells).

Discussion

Lung cancer, either SCLC or non-small cell lung cancer, may be associated a wide spectrum of paraneoplastic syndromes such as secondary endocrinopathies, neurpathies, and autoimmune thrombocytopenic purpura (7,9,10). Because the elevated level of the platelet associated IgG was observed in our case, we could not exclude the possibility of ITP due to SCLC as paraneoplastic phenomenon. However, the patient had no medication for SCLC and ITP for 6 years, and platelet count at admission was within normal range. Therefore, we considered that the ITP in our patient might not have been due to SCLC.

Thrombocytopenia is a frequent comorbid condition in many patients. In some patients, drugs are the cause of low platelet counts. While cytotoxic effects of anti-tumor therapy are the most frequent cause, immune mechanism would also be considered. Demirer et al. reported a patient with SCLC developed autoimmune thrombocytopenic purpura, following a cyclophosphmide, paclitaxel-containig regimen for peripheral blood stem cell mobilization (3). More recently, Mitsuhashi et al showed the autoimmune thrombocytopenia in patients with carcinoma of the uteric cervix developing after cisplatin and radiation therapy (6). In these cases, post-chemotherapy immunologic dysregulation may be a trigger for the development of autoimmune thrombocytopenic purpura. Although there has been no direct

data on showing that it is on immune basis, cytotoxic drugs can cause and recur ITP. We described the case of a SCLC patient with a prior history of ITP who needed the administration of anticancer drugs. To our knowledge, there have been no reports of chemotherapy for SCLC patients with a prior history of ITP in English literature. Our experience shows that it is probably safe to administer chemotherapeutic agents for some cancer patients with prior history of ITP, but it is important to prevent or minimize the toxicities of these chemotherapeutic agents. Fully consideration about the indication of chemotherapy and careful observation are required for such patients.

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Hiroaki Satoh, M.D.,
Division of Respiratory Medicine,
Institute of Clinical Medicine,
University of Tsukuba,
Tsukuba-city, Ibaraki,
305-8575 Japan.
e-mail: hirosato@md.tsukuba.ac.jp