

Biochemical and Biophysical Investigation of Surfactant in Neonatal Gastric Aspirate at Birth



Investigação Bioquímica e Biofísica do Surfactante em Aspirado Gástrico Neonatal ao Nascimento

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Acta Med Port 2013 Jan-Feb;26(1):33-38

ABSTRACT

Background: The optimal approach to detection of surfactant deficiency in the prematurely born infants at birth remains unclear and the decision to apply exogenous surfactant is based mainly on the development of clinical and radiological signs of neonatal respiratory distress syndrome (NRDS).

Objectives: We studied the biochemical and biophysical properties of gastric aspirates (GA) from prematurely born infants with NRDS and healthy full term infants with an aim to find an approachable method for assessment of surfactant maturity at birth.

Material and Methods: Forty-seven newborn infants divided into two groups were enrolled in the study. The first group comprised 34 healthy infants born at term (after 37 weeks of gestation). The second group included 13 premature infants (aged from 26 to 32 weeks of gestation) developing clinical signs of NRDS for which they were treated by assisted ventilation and exogenous surfactant. A biochemical analysis of the protein and lipid content of GA collected at birth was performed. The fatty acid composition of the GA samples was determined by Gas Chromatography-Mass Selective Detector (GS-MSD) analysis. The surface characteristics (equilibrium, maximal and minimal surface tension values) of the GA samples were measured by using the pending drop method. Data were compared between the groups by using Student's t test or Mann-Whitney analysis. Values were considered significantly different if the p value was ≤ 0.05 .

Results: The mean phospholipids' concentration in GA of the premature infants was lower (295.7 $\mu\text{g} / \text{ml}$ vs. 374.5 $\mu\text{g} / \text{ml}$) than in the term infants and the mean protein content was less in GA of the premature babies than the term newborns (574.5 $\mu\text{g} / \text{ml}$ vs. 641.5 $\mu\text{g} / \text{ml}$). The measurement of dynamic surface characteristics of GA showed significantly higher mean values of the minimal surface tension (γ_{min}) in the premature infants – 20.5 m / Nm compared to the term babies - 12.3 mN/m ($p < 0,01$). There was no difference between the equilibrium surface tensions (38 mN/m vs. 38 mN/m) of both groups; The mean values of maximal surface tension (γ_{max}) in GA did not differ significantly between the groups (50.1 mN/m vs. 48.5 mN/m).

Conclusion: Our findings revealed lower phospholipids' and protein concentrations in the GA at birth from premature infants as compared to the healthy term infants. The dynamic surface characteristics of GA had significant differences between the two groups, the minimal surface tension being the most important parameter for evaluation of surfactant maturity. It could be used in the clinical practice for fast surfactant's assessment in the premature infants in regard to administration of exogenous surfactant.

Keywords: Gastric Lavage; Infant, Newborn; Infant, Small for Gestational Age; Pulmonary Surfactants; Respiratory Distress Syndrome, Newborn.

RESUMO

Introdução: A melhor abordagem para detecção de deficiência de surfactante em recém-nascidos prematuros no nascimento permanece pouco clara e a decisão de aplicar surfactante exógeno é baseada principalmente no desenvolvimento de sinais clínicos e radiológicos da síndrome de angústia respiratória neonatal (NRDS).

Objetivos: Estudamos as propriedades bioquímicas e biofísicas do aspirado gástrico (GA) de crianças nascidas prematuramente com NRDS e saudáveis nascidos a termo, com o objetivo de encontrar um método acessível para avaliação da maturidade do surfactante no nascimento.

Material e Métodos: Quarenta e sete recém-nascidos, divididos em dois grupos foram incluídos no estudo. O primeiro grupo composto por 34 crianças saudáveis nascidas a termo (após 37 semanas de gestação). O segundo grupo incluiu 13 recém-nascidos prematuros (idade entre 26 - 32 semanas de gestação) desenvolveram sinais clínicos de NRDS para os quais foram tratados por ventilação assistida e surfactante exógeno. A análise bioquímica da proteína e lipídio da GA coletado no nascimento foi realizado. A composição de ácidos graxos das amostras GA foi determinada pelo detector de cromatografia de massa seletiva (GS-MSD) análise. As características da superfície (de equilíbrio, os valores máximo e mínimo de tensão de superfície) das amostras de AG foram medidos utilizando o método de gota pendente. Os dados foram comparados entre os grupos, utilizando o teste t de Student ou teste de Mann-Whitney análise. Os valores foram considerados significativamente diferentes se o valor de $p \leq 0,05$.

Resultados: A concentração média de fosfolípidos em GA dos prematuros foi menor (295,7 g / ml vs 374,5 mg / ml) do que em crianças a termo e do conteúdo médio de proteína foi menor no GA dos bebês prematuros que o termo recém-nascidos (574,5 ng / ml vs 641,5 $\mu\text{g} / \text{ml}$). A medição das características de superfície dinâmicas da GA mostraram significativamente maiores valores médios da tensão superficial mínima (γ_{min}) nos bebês prematuros - 20,5 m / Nm em comparação com as crianças a termo - 12,3 mN / m ($p <$

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Recebido: 20 de Junho de 2012 - Aceite: 25 de Agosto de 2012 | Copyright © Ordem dos Médicos 2013

0,01). Não houve diferença entre as tensões superficiais de equilíbrio (38 mN / m contra 38 mN / m) de ambos os grupos, os valores médios de tensão de superfície máxima (γ_{max}) em GA não diferiram significativamente entre os grupos (50,1 mN / m vs 48,5 mN / m). **Conclusão:** Os achados revelaram fosfolípidios inferiores e concentrações de proteína na IG ao nascimento de bebês prematuros em comparação com os lactentes saudáveis a longo prazo. As características da superfície dinâmicas da GA apresentaram diferenças significativas entre os dois grupos, a tensão de superfície mínima sendo o parâmetro mais importante para a avaliação da maturidade surfactante. Ele pode ser utilizado na prática clínica para a avaliação rápida de surfactante em recém-nascidos prematuros com respeito à administração de surfactante.

Palavras-chave: Aspirado Gástrico; Recém-Nascido; Recém-Nascido Prematuro; Síndrome de Angústia Respiratória Neonatal; Surfactante Pulmonar.

INTRODUCTION

Alveolar surfactant (AS) is a complex lipoprotein mixture that covers the lung alveoli at the air-liquid interface. Its main function is to reduce the surface tension in exhalation thus preventing the alveolar collapse.¹⁻³ AS components provide alveolar stability by maintaining low surface tension during alveolar area change in the respiration process. The surfactant consists mainly of phospholipids (PL) and proteins. Some of the proteins are specific for AS and play an important role in alveolar stabilization during exhalation as well as in the lung immune defense.⁴⁻⁶ AS insufficiency as well as changes in its optimal biochemical composition result in respiratory activity impairment, most severe of which is the neonatal respiratory distress syndrome (NRDS).

The extreme immaturity including NRDS is a leading cause of the neonatal morbidity and mortality in the developed countries. For this reason a lot of biochemical and biophysical methods and models for investigation of the alveolar surfactant's (AS) structure and properties have been widely developed in the recent decades.

So far the neonatal lung maturity was diagnosed by tests using amniotic fluid, tracheal aspirate, nasopharyngeal aspirate, etc. Some of these methods are traumatic and invasive, others can provide samples with too small quantities, which significantly complicate their analyses. Therefore, it is necessary to look for new diagnostic methods for assessment of surfactant maturity at birth that would be less invasive and painless and that would provide sufficient sample quantity. In search for solution of this problem, we performed a number of biochemical and biophysical analyses of gastric aspirates collected at birth from newborn infants with an aim to find a correlation between the parameters tested and the neonatal surfactant maturity.

MATERIALS AND METHODS

Gastric aspirates: The gastric aspirates were collected in the first minutes after birth by a routine suction of the stomach through a gastric tube and were stored in sterile test-tubes at -20°C . The quantity of each sample was between 2 and 5 ml that sufficed for all biochemical and biophysical analyzes described below.

Biochemical analyzes of gastric aspirates: For determination of protein content in GA Lowry protein assay (Peterson's modification) was used.⁷ The PL' concentration was determined via extraction by the method of Blight and Dyer.⁸ After the extraction, the total PL' content was measured according to the quantity of inorganic phosphorus.⁹

Determination of the fatty acid composition of gastric aspirates by Gas Chromatography-Mass Selective Detector (GS-MSD) analysis: GA samples for the GS-MSD analysis were prepared by the procedure described by W. Christie.¹⁰ The samples dissolved in hexane were analyzed by GC 7890A MS 7000A QQQ Agilent Technologies, USA. A column 'HP Innnowax' 30 m, $250\ \mu\text{m} \times 250\ \mu\text{m}$ was used. The gas phase was helium with constant steam 0.8 ml/min. The analysis was made at temperature of the injector 260°C and mode splitless. The temperature program used was as follows: initial temperature, 150°C without retention, and gradient $4^{\circ}\text{C}/\text{min}$ up to 250°C ; retention at 250°C for 25 min.

Measurements of equilibrium and dynamic surface characteristics of gastric aspirates by the pending drop method:

The pending drop method allows analysis of the surface behavior of small amounts ($50\ \mu\text{l}$) of the tested gastric aspirates. Tensiometer KSV CAM 101 (KSV Instruments Ltd., Finland) was used. The surface tension was determined by using the Axisymmetric Drop Shape Analysis (ADSA). The setup was computer-controlled by a Windows-integrated program, including the ADSA surface tension calculation algorithm. Fifteen minutes were allowed after the symmetric drop of GA formation for adsorption of surface-active molecules at the air-water interface and reaching an equilibrium value of the surface tension, $\gamma_{equilibrium}$ (γ_{eq} , mN/m). By a camera connected to the apparatus series of pictures with different speed (from 60 to 300 pictures per minute) were taken and by ADSA γ_{eq} were determined. After recording this value the drop was subjected to 10-fold compression and decompression by specialized apparatus that imitate the inspiration/exhalation of the lung alveoli during respiration. Thus, its surface changed 5 times, from 100% to 20%. In these dynamic conditions the following surface

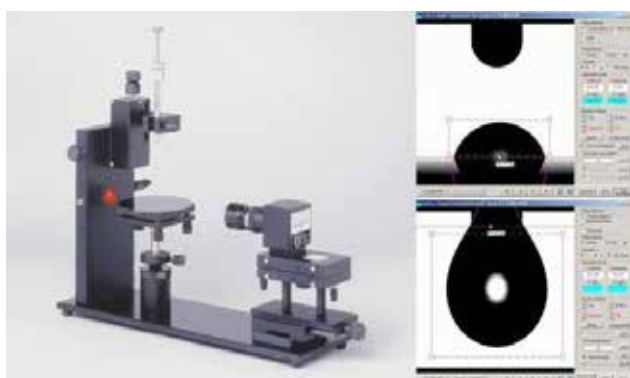


Figure 1: Tensiometer KSV CAM 101 (KSV Instruments Ltd., Finland)

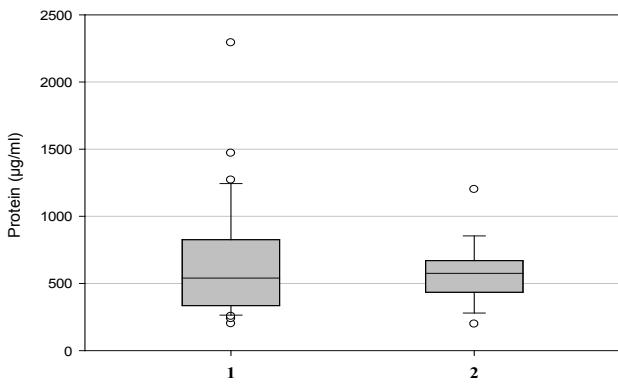


Figure 2 - Protein content (µg/ml) in gastric aspirates from: 1. healthy full term infants; 2. premature infants with NRDS.

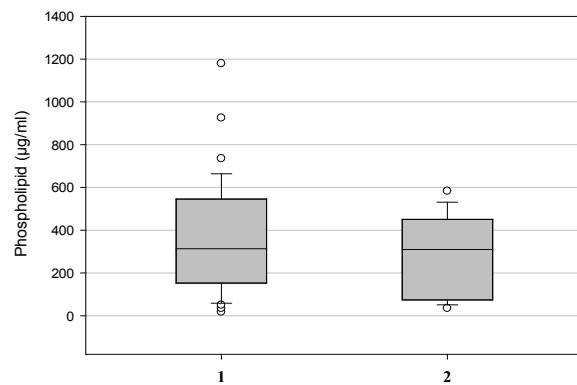


Figure 3 - Phospholipid content (µg/ml) in gastric aspirates from: 1. healthy full term infants; 2. premature infants with NRDS.

parameters were detected: maximal value of the surface tension at 100% drop surface (γ_{max} , mN/m) and minimal value of surface tension at 20% drop surface (γ_{min} , mN/m).

Data analysis

Data were analysed using Student's t test and Mann-Whitney analysis. Descriptive results are expressed as numbers and proportions (%). A *p* value < 0.05 was considered significant.

RESULTS AND DISCUSSION

The GA studied were distributed into two groups: I group – consisted of GA taken from healthy full term infants (34 samples); II group comprised GA of premature infants with NRDS (13 samples).

The protein content of each sample was determined, and the results obtained were summarized for each of the two studied groups (Fig. 2). The median protein content was lower in the premature neonates with NRDS (574.5 µg / ml), as compared to the healthy full term infants (641.5 µg / ml). However, the difference between the mean protein concentrations of the two groups was not statistically significant (*p* = 0.12).

The mean PL' concentration in GA of the premature infants was lower (295.7 µg/ml) than the full term infants (375.5 µg/ml), (Fig. 3). However, the difference between the medians was not significant (*p* > 0.05).

It is known that AS consists of approximately 10% proteins. A part of them originates from serum, i.e. albumin and immunoglobulin G (IgG), presented as nonspecific proteins. However, the major part of the AS protein consists of specific surfactant-proteins. Nevertheless, we consider that the protein concentration of neonatal GA can be easily influenced by different factors associated with swallowing of amniotic fluid, meconium, blood etc. during the delivery. Consequently, this parameter is not reliable for assessment of surfactant maturity at birth.

Although the specific surfactant proteins contribute extremely well to the establishing of AS functions, the phospholipids are the responsible for achievement of low surface tension at the end of expiration. It makes impression that the AS isolated from different kinds of mammalian have

similar lipid composition.¹¹ The phospholipids prevail and they play a major role for optimal functioning of the AS.¹²⁻¹⁶ The main component among them is phosphatidylcholine – 72 - 80%, followed by phosphatidylglycerol – 8 - 12%. Phospholipids as phosphatidylserine, phosphatidylethanolamine and sphingomieline, which are the main components of the cellular membranes, are present in minor quantities in the AS.^{17,18} The neutral lipids make as high as 7 - 10% of the lipids in AS,¹¹ the largest proportion – 80 - 90% consisting of cholesterol. In most mammalian are established small quantities of mono-, bi- and tri-acylglycerols, as well as of free fatty acids, the main of which being the palmitine acid. It is obvious that with the advance of fetal development the PL' concentration is increasing, which is a physiological result of the raising PL' secretion in the fetal lung. Our previous results from analyses of tracheal aspirates of premature infants with NRDS showed that the mean PL concentration in them was around 500 µg/ml,¹⁹ which is much higher than the PL' content in GA. However, unlike the tracheal aspirates the present study of GA did not show a significant difference of PL' content between the two groups. We need more data to be able to find out whether the lower concentration of PL in GA is associated with a risk of development of NRDS.

In this study for first time was performed a fatty acid analysis of the lipids in GA from healthy newborns and premature infants with NRDS (Fig. 4). The palmitine fatty acid (C16:0) has the highest content in GA of the term infants – up to 40% from the total fatty-acid content.

However this value is significantly lower in comparison with the values found in the alveolar surfactant from healthy adults – around 80%.¹³ The decreased content of C16:0 in GA from healthy newborn infants may be due to the presence of amniotic fluid and gastric secretion in addition to the swallowed lung fluid at birth.

The mean percentage of palmitine fatty acid in GA of the premature infants was lower – about 27%, compared to that of healthy term infants (Fig. 4). Besides, the total content of saturated fatty acids with different number of carbon atoms in GA was lower in the premature infants with NRDS, which can explain the lower concentration of PL and respectively the functional deficiency of AS associated with the respira-

tory disease.

The investigations on fatty-acid composition of the particular phospholipids' components of AS show a certain correlation between the fatty-acid profile and the normal biophysical function of surfactant.^{20,21} There is plenty of evidence in the literature that in AS the saturated fatty acids have a larger quantity than the unsaturated ones. The palmitine acid (C16:0) is the prevalent among the saturated fatty acids as its quantity is growing in all phospholipids studied with the increasing of gestational age.^{20,22} Significantly lower content of palmitine acid (C16:0) was observed in premature infants with NRDS or interstitial lung disease compared to healthy infants.²⁰⁻²² It has to be emphasized that the cited data is about the fatty acid profile of AS only and the results are related to the particular phospholipids' components.

In addition to the biochemical analyses, the surface characteristics of the GA samples were determined by the pending drop method. We measured the following surface parameters: equilibrium, maximal and minimal surface tension.

The mean values of equilibrium surface tension in both groups were 38 mN/m (Fig. 5). Obviously the equilibrium surface tension, which was registered in static conditions, is not significant for AS' behaviour *in vivo*, as the physiological

function of alveoli is associated with a permanent change of the surfactant monolayer surface.

In contrast to the equilibrium surface tension the mean values of the dynamic surface characteristics of GA were significantly different between the two groups (Figs. 6 and 7).

The mean value of minimal surface tension (γ_{min}) in GA from the healthy term infants was 12.3 mN / m, while in the group of premature infants with NRDS the median γ_{min} was significantly higher – 20.5 mN / m, $p < 0.01$ (Fig.6).

The minimal surface tension has been proved to be a sensible and informative parameter for evaluation of fetal and neonatal lung maturity.¹²⁻¹⁴ It is well known that in conditions of compression *in vivo*, the AS can achieve very low values of γ_{min} - less than 5mN / m,²³ which is of important significance for prevention of alveolar collapse at the end of exhalation. Our results have also shown, that this parameter can be used as well as with GA samples for a rapid assessment of lung maturity immediately after birth. Besides that, its measurement takes a short time and can be easily fulfilled.

The main property of AS is to reduce the surface tension at the air-liquid interface in the alveoli. The mono- and multilayer films of AS, existing *in vivo*, are characterized with a rapid adsorption by the hypophase, an equilibrium

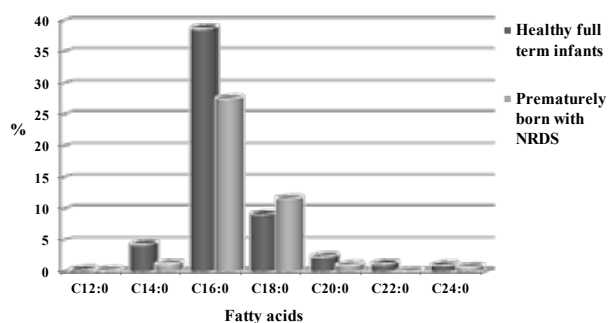


Figure 4 - Content of saturated fatty acids with different number of carbon atoms in GA samples.

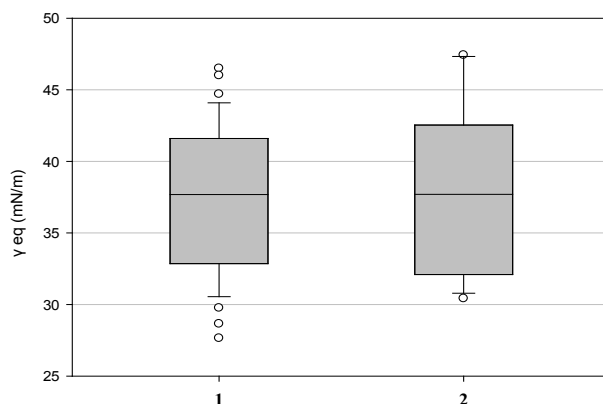


Figure 5 - Equilibrium surface tension values (γ_{eq} , mN/m) in GA from: 1. healthy full term infants; 2. prematurely born with NRDS.

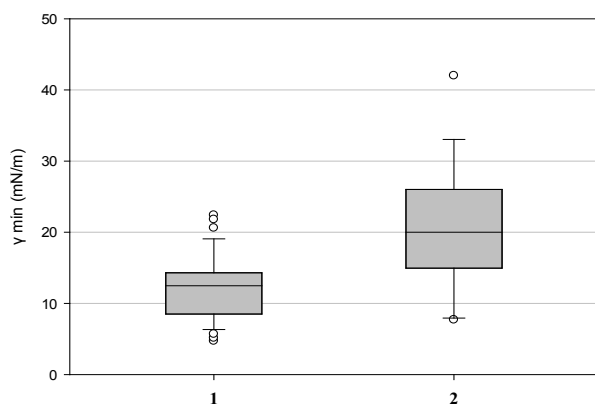


Figure 6 - Minimal surface tension values (γ_{min} , mN/m) in GA from: 1. healthy full term infants; 2. premature infants with NRDS ($p < 0.01$).

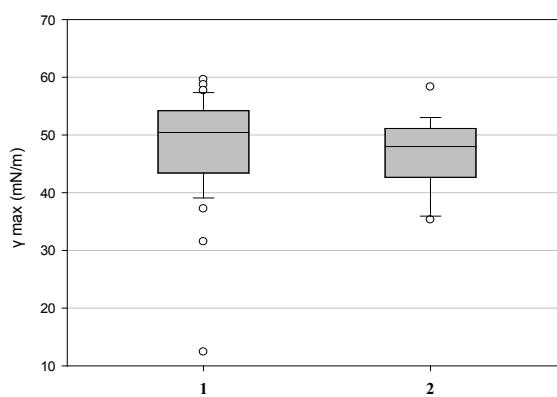


Figure 7 - Maximal surface tension values (γ_{max} , mN/m) in GA from: 1. healthy full term infants; 2. premature infants with NRDS.

value of the surface tension around 25 mN / m in a volume concentration of PL higher than 50 mg / ml and high stability at surface compression.¹⁴⁻¹⁶ Different biophysical and physicochemical techniques and models have been used for investigation of the role of particular surfactant components in the different stages of AS functioning. In the current study we used a relatively new technique for assessment of the biophysical characteristics of AS. It allows to analyze small sample volumes of GA immediately after birth without any additional processing. In addition, it takes relatively short time and its performance is feasible. Our results have shown that the measurement of minimal surface tension in GA could be used as a method for assessment of neonatal lung maturity at birth.

The results obtained have shown that the mean maximal surface tension (γ_{\max}) in GA of the control group – 50.1 mN / m was not significantly different from the mean γ_{\max} in the group of preterm infants with NRDS – 48.5 mN / m (Fig. 7). Unlike the minimal surface tension, we did not observe a similar tendency between γ_{\max} and the development of NRDS.

However, further research is needed to establish whether this parameter is reliable for distinction of surfactant deficiency, respectively NRDS.

CONCLUSION

The biochemical analysis of GA from newborn infants has shown that the differences of the protein as well as the phospholipids' concentration between healthy term and preterm infants with NRDS are not statistically significant. For this reason, at this stage of our knowledge, they may not be used as diagnostic markers for NRDS.

A qualitative and semi-quantitative fatty acids analysis of the lipids containing in the GA samples has been performed by Gas Chromatography-Mass Selective Detector (GS-MSD). Our results have shown that the palmitine fatty acid (C16:0) which is typical of the AS, is detected in highest quantity from total fatty-acids composition. Its proportion is significantly lower in the premature infants with NRDS in

comparison with the healthy neonates born at term.

The biophysical analysis of GA has demonstrated that the values of the equilibrium and maximal surface tension have not shown a correlation with the presence of NRDS. In the same time, the values of minimal surface tension were significantly higher in premature infants with NRDS, compared to the healthy term babies, and were statistically reliable. As with the tracheal aspirates, as well as with the GA studied, this parameter could be successfully used for fast evaluation of surfactant deficiency in the premature infants in regard to administration of exogenous surfactant treatment. However, further analyses of GA samples from newborn infants are needed to implement this method in the clinical practice.

What to expect on this topic in future?

Further research on GA from premature and term newborn infants is expected to create a fast, approachable and precise method for diagnostics of respiratory disorders associated with surfactant deficiency. In addition, the implementation of such method could limit the use of much more invasive techniques for diagnostics of surfactant pathologies performed so far.

ACKNOWLEDGMENTS

The authors thank the neonatal units in the following hospitals and centres for providing samples of gastric aspirates from newborns and data for the study: University Maternity hospital 'Maichin dom' in Sofia, Bulgaria; Maternity hospital "Sheinovo", Sofia, Bulgaria

FUNDING SOURCES

The biochemical and biophysical investigations in this study were supported by a grant for project D002-107/08 of the Ministry of education and science of Bulgaria.

CONFLICT OF INTERESTS

None stated.

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