Clinical significance of multiplex cytokine measurements in immune-mediated disorders

PhD Theses

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Introduction

As the leader of the Central Laboratory Department of Semmelweis University I often face several problems and questions that should be addressed with a systemic approach. I.e. when the questions raised by the clinicians should be answered as a whole using the information obtained from clinical data, laboratory investigations and other diagnostic tests. According to my experience this approach is more established when one should offer an opinion about an immune mediated disorder, the impact of an immune modulation therapy, or the progression of disease. In immune mediated disorders the simultaneous and coordinated action of several components – leukocytes, cytokines, acute phase proteins – leads to the development of characteristic signs and symptoms.

For decades just some specific cytokine or other inflammatory parameters were monitored due to specific methods, high volume of samples and high prices and the complexity of inflammatory response could not be described. The recent development in techniques, however, made possible the simultaneous measurement of several cytokines. Complex systems that are available already in Hungary enable us to obtain a detailed picture about the contribution of inflammatory components to immune mediated disorders.

The aim of my PhD work was to compare cytokine levels measured by different systems and to investigate the alteration of cytokine levels in specific disorders. As the intensity of systemic inflammation and cytokine levels are largely affected by free radical reactions and, through free radical generation, trace element levels, I also searched for an association between cytokine levels, antioxidant defence and serum / tissue trace element levels.

The availability of our methods used in these investigations (e.g. multiplex determination of cytokine levels, measurements of trace element levels, investigation of free radical reactions) is limited just for some specific laboratories in Hungary. As these methods are particularly expensive, clinical data are urgently needed to decide their diagnostic relevance. Therefore clinical data should be collected to determine the direction that should be followed during the development of laboratory techniques. I hope that our results and the experience obtained during my PhD work will help this work.

Background

Cytokines, these 8 – 40 kDa small proteins are responsible for the communication between immune cells. The majority of cytokines are not produced normally; their synthesis is triggered by specific factors, i.e. cell stressors (such as UV radiation, heat, hyperosmolarity). After their synthesis cytokines are instantly released and provide an immediate effect.

Cytokines increase or decrease the activation, proliferation and differentiation of immune competent cells, hereby regulating immune processes, the intensity and duration of immune response, synthesis of antibodies and production of further cytokines. All the immune cells is able to produce cytokines and possesses cytokine receptors. Cytokines exert their immune modulatory activities through in an autocrine or paracrine or pleiotropic or sometimes endocrine way.

Data regarding the contribution of cytokines to physiological processes and disorders are collected for more than two decades. However, there are several contradictions, partly due to methodological problems.

There are several difficulties with the determination of cytokines. Most important ones are:

(1) Cytokines with the same name may be present in different forms (i.e. as monomers and polymers) in the body. Some of them consisted of different parts that are produced by different cell types. The chemical structure is not always well characterized. As cytokines are proteins with several possible epitopes, the sensitivity and specificity of immune analytical methods used for their determination are largely influenced by the technology of their production.

(2) The majority of cytokines are not detected in normal state as their level is very low.

(3) Cytokine levels dramatically increase after activation of immune cells. However, this change is transient. Kinetics of different cytokines may largely differ.

(4) As cytokines exert their effects as elements of a complex system, the clinical information that the measurement of a particular cytokine level may provide is limited.

These methodological challenges should be adequately and sufficiently answered by the clinical laboratory. The goal of my work was to collect and evaluate relevant data about cytokine patterns in different disorders in order to determine their clinical utility.

Aims

The first step during my PhD work was to introduce and compare **two multiplex cytokine measurement systems** to our laboratory.

The goals of my work were to characterize **cytokine profiles** in **different immune mediated disorders** and to investigate the possible association between cytokine patterns, **free radical reactions** and **trace element (metal ion) levels**.

Methodological issues

1. Comparison of cytokine measurement techniques: comparison of cytokine levels measured by two multiplex systems (Biochip® and Bioplex®), and IL-6 levels measured individually; establishment of correlation between measured values.

Perinatology

2. Evaluation of the impact of hypothermic treatment on systemic cytokine levels in post-asphyxiated neonates.

3. Investigation of the impact of endogenous cortisol levels on systemic inflammatory cytokine levels in preterm infants.

4. Evaluation of serum cytokine patterns in septic neonates and their comparison with procalcitonin and CRP levels.

Liver disorders

5. Investigation of the impact of moderate red wine consumption on the systemic levels of cytokines, trace elements and redox status.

6. Characterization of systemic cytokine patterns in cirrhosis due to different causes.

7. Investigation of cytokine and trace element levels in Wilson's disease: evaluation whether there is a specific cytokine pattern in Wilson's disease with neurological symptoms

Other disorders

8. The investigation of the impact of antioxidant supplementation on redox status, trace element levels and systemic cytokine levels in patients with metastatic prostate cancer.

9. The investigation of the association between Malnutrition Inflammation Score (MIS) used for the recognition of malnutrition inflammation complex syndrome (MICS) in renal patients and the level of inflammatory cytokines.

Patients and methods

We enrolled the following patient groups into our studies

Patient groups	Time of sampling	Other analytes tested
20 healthy individuals	Once	-
19 asphyxic neonates (of those, 10 were treated with hypothermia)	Postnatal hour 6, 12. 24 and 72	cortisol
40 preterm infants with a birth weight under 1500 grams	Postnatal day 0. 1, 3 and 7	cortisol
41 septic preterm and term neonates	Before antibiotic treatment, then on Day 1. and Day 2. on therapy	CRP, procalcitonin Cortisol
20 healthy young adults	At study entry, then 1 month after moderate red wine consumption	Trace element (metal) levels, redox status
75 patients with compensated liver cirrhosis (due to alcoholic [n=36], primary biliary [n=25] and HCV infection [n=14])	Once	-
50 Patients with Wilson's disease on D-penicillamine treatment (of those, 24 presented with neurological symptoms)	Once	Trace element (metal) levels, redox status
18 patients with metastatised prostate cancer	At study entry, then after 1 month of consumption of beetroot extraction	Trace element (metal) levels, redox status
993 patients after renal transplantation	Once, when obtaining the MIS value	Malnutrition inflammation score (MIS)

For investigational purposes, blood samples (5 ml and 2 ml from adult and neonatal subjects, respectively) were taken.

Multiplex serum cytokine levels were measured with Biochip and Bioplex systems.

Individual cytokine levels were determined with Roche immunoassay and ELISA.

Trace elements (metals) were measured from plasma with plasma emission spectrometry.

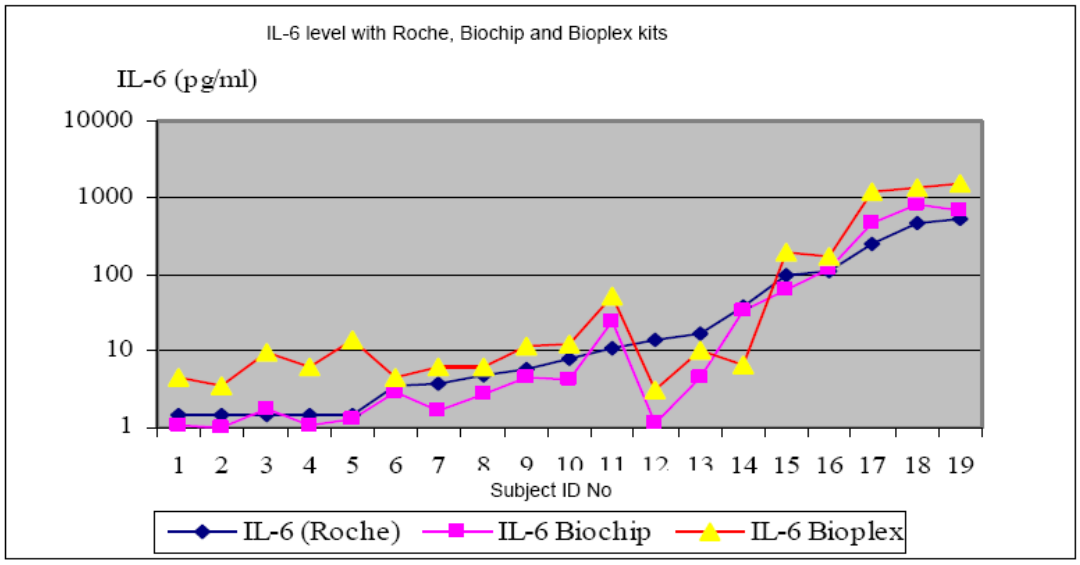
For the investigation of redox status, H-donor (HDON) activity and total scavenger capacity (TSC) were measured.

The malnutrition-inflammation score was determined with a questionnaire validated previously in patients with chronic renal disease.

Results

Comparison of cytokine measurement techniques

IL-6 levels measured in different systems strongly correlated.



IL-6 levels measured with Roche ELISA kit, Biochip and Bioplex system

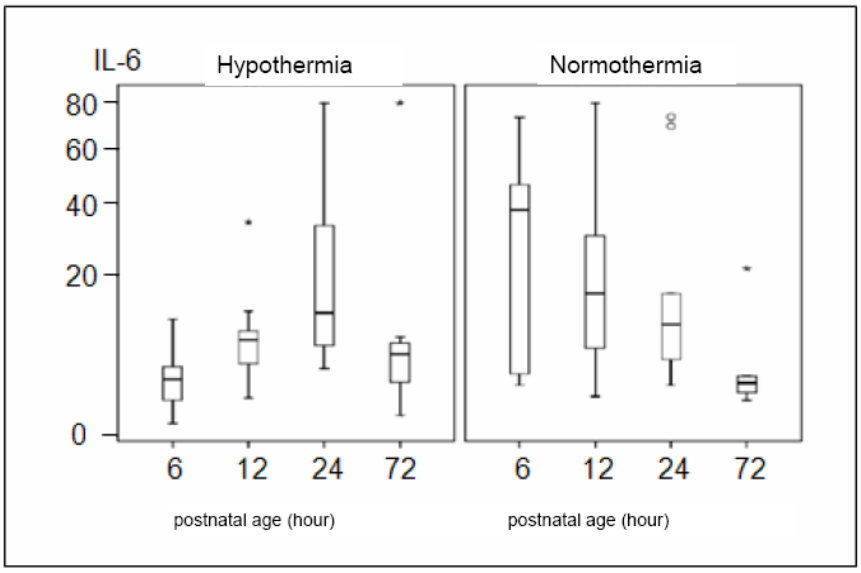
IL-6 levels measured with Bioplex system were almost significantly higher than those measured with Roche ($p = 0.07$), or Biochip ($p = 0.06$) system. The strength of correlation between cytokine levels measured with Bioplex and Biochip systems varied largely ($r = 0.28–0.97$).

	r value		**r value**
IL-6	0.97	**TNF-α**	0.48
IL-2	0.35	**IL-4**	0.28
IL-8	0.68	**IFNγ**	0.83
IL-10	0.98		

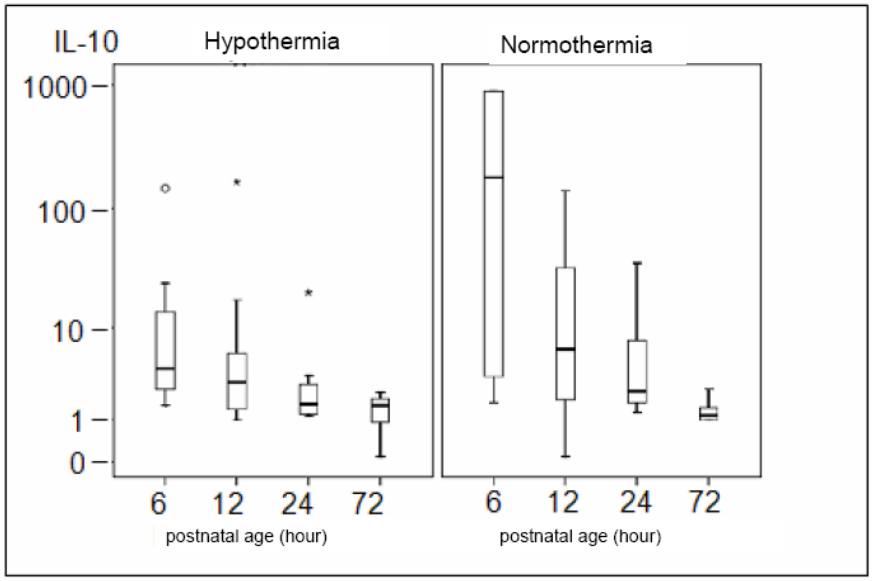
Strength of correlation between cytokine levels measured with Bioplex and Biochip systems

Impact of hypothermia on cytokine level kinetics in postasphyxic neonates

Between 6th and 72nd postnatal hour we observed a significant effect of postnatal age on proinflammatory cytokine levels ($p = 0.049$), and an interaction between postnatal age and hypothermia ($p = 0.049$). For hypothermia, we observed a significant effect on antiinflammatory cytokines ($p = 0.023$). In both groups IL-4/IFN-γ ratios increased significantly between 6th and 72nd postnatal hour ($p < 0.001$).



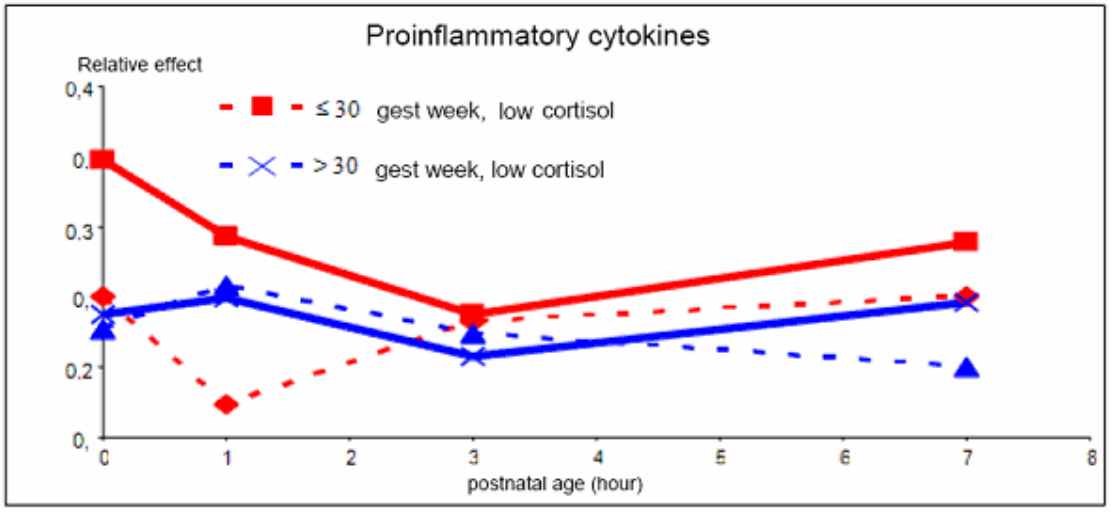
IL-6 levels in neonates treated with hypothermia or normothermia (median, interquartile range).



IL-10 levels in neonates treated with hypothermia or normothermia (median, interquartile range)

Impact of endogenous cortisol levels on cytokine kinetics

We observed higher pro-inflammatory cytokine levels in preterm infants with cortisol levels lower than the median ($p < 0.0001$). This phenomenon is more characteristic in infants with lower gestational age.



In postasphyxic neonates we demonstrated that the level of pro-inflammatory cytokines is increased, while the level of anti-inflammatory cytokines is decreased in neonates on hypothermia and with low cortisol levels. In neonates on normothermia no difference between neonates with low and with high cortisol levels was observed.

Perinatal sepsis and cytokine patterns

Each neonate (who was septic according to procalcitonin and/or CRP levels) presented with an increased IL-6 level. In 6 of the 15 neonates with an increased IL-8 level procalcitonin and CRP levels were within the healthy reference range. 95% of neonates with signs and symptoms of perinatal sepsis presented with an IL-8 level above 90 pg/ml and/or procalcitonin level above 2.5 µg/l and/or CRP- level above 3 mg/l on the first day of sepsis.

Gender dependent effect of red wine consumption

After one month of red wine consumption IL1α and IL2 cytokine levels and VEGF levels increased, while those of IL6 decreased in women. In men, no significant alteration was observed in any cytokine levels during the study.

Cytokine level	Women		Men	
(pg/ml)	Baseline	At the end of the study	Baseline	At the end of the study
IFN-γ	1,5 [0.0 – 3,9]	2.0 [0.0 – 6,0]	2.2 [0.0 – 4,7]	2.0 [0.0 – 4,4]
IL-10	0.8 [0.0 – 1.7]	0.7 [0.0 – 1.2]	1.4 [0.0 – 2.3]	1.1 [0.0 – 3.0]
IL- 1α	0.4 [0.0 – 0.8]	0.9 [0.5 – 1.3]**	0.9[0.0 – 1.8]	0.8 [0.0 – 1.7]
IL- 1β	1.7 [0.0 – 4.3]	1.0 [0.0 – 2.3]	1.7 [0.0 – 4.5]	0.7 [0.0 – 2.0]
IL- 2	4.8 [0.0 – 9.8]	7.8 [5.6- 12.2]*	4.4 [0.0 – 6.9]	5.4 [0.0 – 7.8]
IL- 4	4.3 [2.1 – 7.0]	3.8 [2.1 – 6.8]	4.4 [2.4 – 8.5]	3.3 [0.0 – 6.6]
IL- 6	1.7 [0.6 – 4.8]	1.2 [0.0 – 3.7]*	1.0 [0.0 – 1.7]	1.1 [0.0 – 4.6]
IL- 8	21.1 [7.5 – 30.6]	20.0 [7.1 – 31.8]	22.1 [9.4 – 35.0]	13.7 [5.5 – 24.0]
TNF-α	10.3 [4.9 – 20.8]	9.9 [7.5 – 14.2]	8.1 [4.9 – 10.2]	12.1 [5.5 – 30.0]

*** p<0.01 * p<0.05*

In women, red cell Ca, Mg, Pb, Sr and Zn levels and Zn/Cu ratio at the end of the study were lower than measured at the baseline. To the contrary, Al, Ca, Li, Pb and Sr levels decreased in men.

Simultaneously with trace element alterations we also observed some changes in antioxidant defence. Baseline TSC and HDON parameters were significantly different in men and women. In women, HDON and plasma reducing capacity increased, while TSC reflecting free radical generating capacity of plasma and red cells decreased by the end of the study ($p<0.001$). In men, HDON and plasma reducing capacity increased, while plasma TSC decreased. Several significant correlations were demonstrated between cytokine levels, redox status and trace element levels.

Cytokine patterns in cirrhosis

IL-6, the acute phase protein produced in the liver is significantly increased in any type of cirrhosis. However, cytokine patterns measured in primary biliary cirrhosis, HCV infection and chronic alcohol consumption were significantly different.

Cytokines (pg/ml)	Healthy reference group	Alcoholic cirrhosis	Primary biliary cirrhosis	Cirrhosis due to HCV infection
	n = 26	n = 36	n = 35	n = 14
IFNγ	1,78 [0.0 - 3,94]	2.18 [1,62 - 4,74]	2.05 [0.0 - 7,14]	1,60 [0.0 - 3,48]
IL-10	1,08 [0.0 - 2.07]	1,20 [0.12 - 2.71]	0.47 [0.0 - 3,32]*	1,08 [0.42 - 2.86]
IL-1α	0.64 [0.0 - 1,34]	0.62 [0.0 - 1,41]	0.25 [0.0 - 2.03]	0.57 [0.0 - 1,82]
IL-1β	1,57 [0.0 - 4,32]	1,17 [0.0 - 3,31]	0.72 [0.0 - 3,21]	1,57 [0.0 - 2.97]
IL-2	4,18 [0.0 - 6,83]	5,50 [3,95 - 13,2]	4,88 [0.0 - 9,12]	8,18 [3,24 - 13,8]*
IL-4	4,56 [2.15 - 6,97]	4,93 [2.24 - 10.3]	2.39 [0.0 - 9,04]	5,90 [2.26 - 11,4]
IL-6	1,51 [0.74 - 4,89]	10.8 [1,02 - 24,7]*	5,72 [0.0 - 17,6]*	4,80 [0.0 - 9,46]
IL-8	25,2 [6,84 - 48,1]	49,2 [7,72 - 138]	159 [14,9 - 305]*	17,7 [3,88 - 7,2]
TNFα	7,45 [2.54 - 14,4]	4,58 [2.15 - 7,51]	11,4 [0.0 - 31,3]*	4,81 [1,94 - 8,62]

** p< 0.05 compared to the healthy reference group*

In patients with cirrhosis due to HCV infection and in those with primary biliary cirrhosis IL-2 levels, and the pro-inflammatory IL-8 and TNFα levels, respectively, were higher than those measured in the healthy reference group. The levels of anti-inflammatory IL-10 cytokine were lower in patients with primary biliary cirrhosis compared to those in other groups.

Wilson's disease and cytokines

Our results indicated that the patients with Wilson's disease and treated with D-penicillamine are presented with altered trace element levels and just Cu levels are normalized. In patients with neurological symptoms Cr, Li, Ni and Pb levels were significantly higher compared to those without neurological symptoms

Trace element	Healthy reference values (n = 31)	Wilson's disease	
		Without neurologic symptoms (n = 16)	With neurologic symptoms (n = 18)
Al*	0.270 ± 0.088	0.680 ± 0.231	0.626 ± 0.180
Ba*	0.007 ± 0.002	0.695 ± 0.108	0.779 ± 0.150
Ca*	1,451 ± 0.369	3,460 ± 1,168	3,540 ± 1,023
Cr**	0.139 ± 0.048	0.046 ± 0.029	0.231 ± 0.047
Cu	0.030 ± 0.012	0.061 ± 0.027	0.076 ± 0.036
Fe*	15,03 ± 3,38	29,55 ± 5,40	31,52 ± 6,07
Li*,**	0.110 ± 0.032	0.157 ± 0.015	0.198 ± 0.059
Mg*	0.928 ± 0.176	1,896 ± 0.385	1,764 ± 0.293
Ni**	0.280 ± 0.038	0.089 ± 0.057	0.357 ± 0.245
P*	6,012 ± 2.614	18,762 ± 5,869	21,145 ± 5,773
Pb**	0.053 ± 0.024	0.082 ± 0.055	0.175 ± 0.083
Zn	0.471 ± 0.170	0.750 ± 0.423	0.575 ± 0.193

** significant compared to healthy individuals (p<0.05)*
*** significant between two patient groups with Wilson disease (p<0.05)*

Based on routine laboratory parameters patients with Wilson's disease with and without neurological symptoms were comparable. Antioxidant parameters and cytokine levels did not differ significantly either; however. IL-6 and IL-8 levels were higher than normal in both groups ($p<0.05$, and $p<0.001$).

Cytokine (pg/ml)	Healthy reference values n=26	Wilson's disease			
		neurological symptoms	hepatic involvement	neurological + hepatic involvement	symptom free
		n=22	n=6	n=7	n=17
IL-6	1.51 [0.74 - 4.89]	4.81 [0.76 - 14.98]	7.87 [0.66 - 29.2]	4.73 [1.89 - 7.72]	6.77 [0.83 - 35.4]
IL-8	25.2 [6.84 - 48.1]	89.1 [11.0 – 231]	62.0 [10.0 - 206]	81.0 [18.0 - 239]	41.1 [17.2 - 84.1]

The alteration of cytokine patterns in patients with prostate cancer before and after beetroot extraction treatment

The level of the majority of investigated cytokines (IFNγ, IL-1α, IL-1β, IL-2. IL-6, IL-8) somewhat decreased (although to a non-significant extent) or did not alter (TNF-α). While VEGF levels remained unaltered, the EGF levels almost doubled ($p=0.003$).

Parameters	**Treatment with beetroot extraction**	
	before	**after**
IFNγ	4.28 (0.04 - 9.52)	2.21 (0.3 - 4.18)
IL-1α (pg/ml)	0.54 (0.12 – 2.33)	0.35 (0.19 – 0.52)
IL-1β (pg/ml)	1.18 (0.60 – 4.33)	0.42 (0.10 – 0.92)
IL-2 (pg/ml)	7.8 (4.4 – 15.5)	5.5 (2.2 – 13.3)
IL-6 (pg/ml)	14.2 (4.2 – 36.3)	5.6 (2.3 – 12.1)
IL-8 (pg/ml)	31.2 (17.1 – 223.4)	25.3 (12.2 - 54.9)
TNF-α (pg/ml)	3.41 (1.6 – 7.5)	3.50 (2.52 – 4.48)
VEGF (pg/ml)	272 (146 - 388)	282 (120 - 442)
EGF (pg/ml)	59 (19 - 100)	110 (52 - 170)*
PSA (ng/ml)	93 (21 - 210)	133 (17 - 320)
fPSA (ng/ml)	20.2 (10.2 - 68.3)	30.6 (6.5 - 86.7)

* *$p<0.05$*

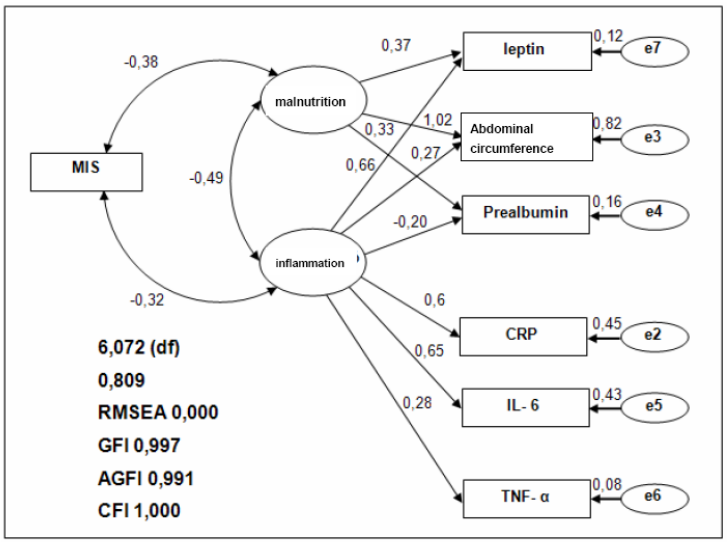
Malnutrition-inflammation complex syndrome in patients after renal transplantation

The mean BMI value was 27 ±5 kg/m^2. Abdominal circumference was higher in men than in women (103±13 cm vs 93±14 cm; $p<0.001$ and 36.5±7.6 mg/dl vs 32.1±6.8 mg/dl; $p<0.001$). Major parameters reflecting malnutrition and inflammation:

	Total patient population (n=993)	Men (n=569; 57%)	Women (n=424; 43%)	P value
MIS (median (IQR); mean)	3 (3); 3.6	3 (3); 3.2	3 (3); 4.1	<0.001
Body mass(kg) (mean ± SD)	75±16	81±15	68±13	<0.001
Abdominal circumference (cm) (mean ± SD)	99 ± 14	103 ± 13	93 ± 14	<0.001
BMI (kg/m2) (mean ± SD)	27 ± 4.9	27.2 ± 4.6	26.7 ± 5.2	NS
CRP (mg/l) (median (IQR))	3.1 (5.4)	3.1 (4.8)	3.4 (5.8)	NS
Albumin (g/l) (mean ± SD)	40±4	41±4	40±4	<0.001
Pre-albumin (g/l) (mean ± SD)	0.346 ± 0.076	0.36.5 ± 0.07.6	0.32.1 ± 0.06.8	<0.001
Interleukin-6 (ng/l) (median (IQR))	2.09 (2.37)	2.04 (2.02)	2.15 (3.20)	NS
Leptin (µg/l) (median (IQR))	15.1 (25.3)	10.70 (15.44)	28.04 (40.06)	<0.001
TNF-α (ng/l) (median (IQR))	2.06 (1.34)	2.10 (1.31)	1.95 (1.34)	NS

The malnutrition-inflammation score (MIS) significantly correlated to the level of acute phase proteins, renal function and IL-6 and TNF-α cytokine levels.

Based on observed relationships a structural equation model was developed with factors having an impact on MIS.



Theses

Comparison of cytokine measurement methods

I. Cytokine levels determined may depend largely on techniques used, therefore the laboratory should assess healthy reference range for each specific technique.

Clinical studies

Perinatal disorders

II. The level of proinflammatory cytokines may decrease with advancing age and hypothermic treatment in postasphyxiated neonates.

III. The level of pro-inflammatory cytokines is increased in preterm neonates and postasphyxiated term neonates with lower than median cortisol levels.

IV. The diagnostic process of perinatal sepsis may be improved by the addition of IL-8 measurement to procalcitonin and CRP measurements in blood sampled taken before the initiation of antibiotic therapy.

Liver diseases

V. The gender-dependent difference in alcohol induced hepatopathy may be attributed, at least partly, to the increased cytokine production with chronic alcohol consumption. Red wine consumption may have an impact on the level of some trace elements and antioxidant defence.

VI. In liver cirrhosis caused by chronic ethanol consumption, HCV infection and primary biliary cirrhosis (PBC) the IL-6 levels are increased. PBC and HCV-induced cirrhosis are characterized by high IL-8 and IL-2 levels, respectively.

VII. In patients with Wilson's disease and treated with chelating agents a systemic inflammation is present, supported by high IL-6 and IL-8 levels. Neurological signs and symptoms are independent of cytokine levels. The levels of a number of trace elements are increased that may contribute to complications.

Prostate cancer

VIII. The level of pro-inflammatory cytokines decreases after consuming a specific antioxidant nutrient. The treatment may increase the level of some growth factors that warrants caution in patients with prostate cancer.

Patients after renal transplantation

IX. Based on general status, IL-6 and TNF-α levels the malnutrition-inflammatory score reflects reliably the malnutrition-inflammation complex syndrome in this population.

List of publications

Papers related to PhD Theses

1. **Bekő G**, Hagymási K, Szentmihályi K, Bányai ES, Osztovits J, Fodor J, Fehér J, Blázovics A. (2009) Sex-dependent alterations in erythrocyte trace element levels and antioxidant status after a month of moderate daily red wine consumption. Eur J Gastroenterol Hepatol. Sep 25. [Epub ahead of print], IF: 2.08
2. **Bekő G**. (2008) Biochip technika a labordiagnosztikában, citokinmérések. Orvosképzés; 4: 309-312
3. **Bekő G**, Krkos K, Rácz K Patócs A, Tulassay Zs. (2008) A szérum inzulinszerű növekedési faktor-1 (IGF-1) referenciatartományának vizsgálata. Magyar Belorvosi Archivum 5: 400-405.
4. Gyarmati B, **Bekő G**, Szalay B, Cseh A, Vásárhelyi B, Treszl A: Maternal cytokine balance on 3rd postpartum day is not affected by the mode of delivery after healthy pregnancies. The Journal of International Medical Research (elfogadva 2009 szeptember) IF: 0.821
5. Molnár MZ, Czira M, Ambrus C, Szeifert L, Szentkirályi A, **Bekő G**, Rosivall L, Remport Á, Novák M, Mucsi I. Anemia is associated with mortality in kidney-transplanted patients-a prospective cohort study. Am J Transplant. 2007 818-24, IF: 6,423
6. **Bekő G**, Treszl A, Róka A, Vásárhelyi B, Mészáros G, Tulassay T, Azzopardi D, Szabó M.(2009) Changes In Serum Cytokine Levels In Normothermic And Hypothermic Infants After Perinatal Asphyxia. Pediatric Research (revízió alatt)
7. Molnár M. Z, Keszei A, Czira M. E, Rudas A, Újszászi A; Háromszéki B, Kósa J. P, Lakatos P, Sárváry E, **Bekő G**, Fornádi K, Kiss I, Remport Á, Novák M, Kövesdy Cs. P, Kalantar-Zadeh K, Mucsi I. Validation of the Malnutrition-Inflammation Score in a large cohort of kidney transplantrecipients. American Journal of Kidney Disease 2009 (revízió alatt)

Papers published in peer reviewed books:

1. **Bekő G**, Ostovits J, Visnyei Zs, Szalay F, Sátori A, Blázovics A, Szentmihályi K. (2009) Significant difference in the concentration of Special metal elements in Wilson's disease with different symptoms during penicillamine treatment Trace elements in the food chain 3: 31-35. ISBN 978-963-7067-19-8
2. Nyirády P, Blázovics A, Romics I, May Z, Székely E, **Bekő G**, Szentmihályi K. Microelement concentration differences between patients with and without prostate

adenocarcinoma Trace elements in the food chain 2009;3 :26-30. ISBN 978-963-7067-19-8

Referable abstracts and lectures

1. **Bekő G**, Osztovits J, Visnyei Z., Szalay F., Sátory A,. Blázovics A, Szentmihályi K. Significance of measurement and difference of special metal elemens in Wilson's disease during penicillinamin treatment. Clin Chem Lab Med 2009;47 Special Supplement PP s1-s409
2. **Bekő G**, Hagymasi K, Szentmihalyi K Biro E, Osztovits J, Szalay F, Bárkovits S, Blazovics A. (2008) How can nutritional factors modify the cytokine pattern and redox parameters in alcoholic drink consumption women? Clin Chem Lab Med, 46, S351
3. **Bekő G**., Bíró E., Kovács M., Mátrai B. Újabb citokin mérési módszerek a labordiagnosztikában A citokin és növekedési faktor panelek értéke Klin. Kísérl. Lab. Med. 2008 34: 41.
4. Novák M., Fehér A., Nobilis A., Olajos F., **Bekő G**. Újszülöttek és koraszülöttek prokalcitonin és C-reaktív protein szintjének követése az élet korai szakaszában Klin. Kísérl. Lab. Med. 2008 34: 67.
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