Summary report

on OTKA grant 76316

Prediction of perinatal complications in the preterm neonate: lymphocyte activation and biomarkers.

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Background

The perinatal complications threatening the preterm neonate are the consequence of a complex pathomechanism. Major elements include infection, immature and/or unbalanced immune system and the immaturity of the wholy body system. The major aim of our work was to investigate the dynamic changes of adaptive immune system during the early postnatal period and their relationship to perinatal complications.

For this purpose we applied a complex approach.

We enrolled a cohort of 80 very low birth weight neonates who are at the highest risk for perinatal complications.

We developed sensitive micromethods for flow cytometer that provided an opportunity to test the prevalence of the individual members of adaptive immunity and their major regulator cells in a so small volume of blood that does not present a burden for the study participants.

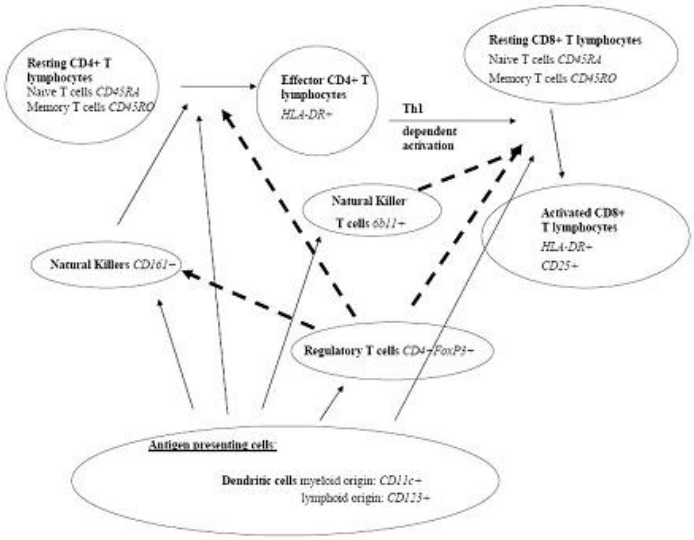

Figure 1. Lymphocyte activation parameters and the surrounding cellular network. *CXCR3* and *CCR4* antibodies are used as surrogate markers of Th1 and Th2 cells, respectively. Continuous line indicates activation, while dotted line indicates inhibition.

We also applied micromethods to monitor the simultaneous alteration of cytokine milieu.

Our methodological achievements also provided an opportunity to investigate the immunological changes in several other conditions that share some similar characteristics in immune phenotype to that

Perinatal alterations of adaptive and innate immunity in the preterm neonate

[Manuscript is under preparation]

The population of preterm neonates cannot be regarded as a homogenous group. They differ in term of maturity (born between 24th and 36th weeks of gestation), the presence of co-existing disorders and the cause of premature birth. About 70 per cent of preterm infants born with a birth weight under 1500 grams are estimated to have a fetal inflammatory response syndrome (FIRS), while the remaining part of them is born due to other factors including maternal preeclampsia, premature rupture of membranes, congenital malformations etc.

The process culminating in premature birth determines the neonate's susceptibility to postnatal complications. The contribution of the exaggerated inflammatory status to periventricular leukomalacia and bronchopulmonary dysplasia is increasingly acknowledged. Indeed, a large body of evidence support that the early exposure of neonatal organs to high IL-1 and IL-6 concentrations disturb the process of postnatal adaptation and result in an uncontrolled inflammatory response that finally leads to the damage of brain and lung tissues. The published reports investigated the association between individual inflammatory markers measured at specific postnatal time points and the risk of postnatal complications in order to find a specific marker that helps the identification of the affected individual. The majority of these studies, however, aimed to test the different soluble and cellular elements of the immune system at specific time point and the data regarding the possible fluctuation of inflammatory markers during the early postnatal period in preterm neonates are scarce. The primary aim of our study was to describe the alteration of cytokine levels, and prevalence of T-cells and some cellular elements of innate immune system during the first postnatal week and their association with the neonates' major clinical characteristics (i.e. gestational age, postnatal age, maternal steroid therapy and presence of FIRS at birth).

Patients and methods

72 preterm neonates born with a birth weight under 1500 grams between January, 2006 and December, 2009 have been enrolled to the study. Prior the first sampling written informed consent was obtained from the mother.

0.5 ml of heparinized blood samples were collected at birth (from cord blood), then in Hour 24, Hour 72 and Hour 168 hour after birth. Blood specimens were centrifuged at 2000 rpm for 5 minutes and the plasma was used for the determination of the level of 17 cytokines (macrophage

inhibitory protein-1 (MIP1); macrophage cationic protein-1 (MCP1); interleukin (IL)-17; IL-13; IL-12; IL-10; IL 8; IL 7; IL 6; IL 5; IL 4; IL 2; IL 1B; interferon-gamma (IFN-γ); granulocyte-macrophage colony stimulating factor (GM-CSF); granulocyte colony stimulating factor (GCSF) and tumor necrosis factor-alpha (TNF-α) with a Bioplex System. To assess adrenal function serum cortisol levels were also measured with a a commercially available Roche kit.

The pellets were resuspended with 1 ml of phosphate buffer saline, divided into four aliquots and used for the determination of the prevalence of different T cell subsets and innate immune cells. The following cell surface markers-fluorescent dyes conjugates were used for the identification of CD4+ and CD8+ T-cells: CCR4-PE; CXCR3-APC (surrogate markers for Th1 and Th2 cells, respectively); CD25-FITC; CD62L-PE-Cy5; HLADR-PerCP (early, intermediate and late activation markers, respectively); CD45RA-FITC and CD45RO-PE (markers of memory and naïve cells, respectively); and for the assessment of cells of innate immunity including CD3-PerCP^{-}-CD161-APC (natural killer cells, NK), CD3^{+}-CD161-APC (natural killer T cells, NKT), CD3-PerCP^{+}-6b11-PE^{+} (invariant NKT cells, iNKT), Lin1-FITC^{-}-HLA-DR-PerCP^{+} (dendritic cells, DC), CD11c-APC (myeloid DC, mDC), and CD123-PE (plasmocytoid DC, pDC). Fluorescent antibodies were obtained from BD (Beckton-Dickinson) and used according to manufacturer's instructions. The measurements were done on a FACS Aria (BD) instrument.

Statistical analysis

After the recruitment of preterm neonate population it became clear that the prevalence of perinatal complications (including BPD, periventricular leukomalacia, IRDS and sepsis) was too low and, therefore, this sample size was insufficient to describe the possible contribution of the investigated elements to each perinatal complication. Therefore we modified our primary aim and, instead, we made efforts to describe the characteristic kinetics of inflammatory elements during the early postnatal period. We tested whether the kinetics of these elements is affected by maternal steroid therapy (given to prevent IRDS), the neonate's adrenal function (characterized by cortisol levels), postnatal age and baseline increased IL-6 levels (suggesting chorioamnionitis, CA).

A mixed model approach was used to test the impact of independent variables (gestational age, postnatal age at sampling, maternal steroid use, cortisol level and CA) on plasma cytokine levels, immune phenotype and calculated ratios (i.e. IL-4/ IFN-γ; IL-10/IL-6 and CD4 CD45RA/CD45RO).

For gestational age, three categories were defined (i.e preterm neonates born at g.w. ≤26, >26 – ≤28 and >28).

For cortisol levels, neonates were classified as neonates with 'low' cortisol (i.e. cortisol levels below the median at three time points or below the median in cord blood sample and another sample) and as

neonates with ‘high’ cortisol levels. IL-6 levels above 10 pg/ml in cord blood were used as surrogate marker for chorioamnionitis.

We performed two series of analysis with different sets of independent variables. First, we tested the overall effect of each of independent variables enlisted above on dependent parameters. Then we did the same analysis separately in neonates with and without CA. A p level below 0.05 was used as the level of significance.

Results

Table 1a -c below summarize the results of our analysis. The individual measurement data are available on request.

Table 1.a Major determinants of investigated parameters in the whole population of preterm neonates (n = 72). Just p values suggesting a significant impact are presented. 'Blue', 'red' and 'grey' colours indicate antiinflammatory, proinflammatory and unknown effect on inflammation, respectively. + or - indicates the proportional or inverse relationship (at gestational categories and postnatal age, three signs of polarity are given). For abbreviations see text

	gest. age category	**age (post-natal)**	**cortisol**	**maternal steroid**	**CA**
Cytokines					
IL-10/IL-6		-++ 0,03			- 0,03
IL4/IFNG					
MIP1					
MCP1					+0,006
IL-17					
IL-13	0,04				+0,0000
IL-12		--+0,02			+0,0000
IL-10	---0,03				+0,0073
IL-8					+0,03
IL-7					
IL-6					+0,000
IL-5	--+0,04				+0,000
IL-4	-++0,02				
IL-2				+0,02	
IL-1B					+0,02
IFN-G					
GM-CSF					-0,01
GCSF					+0,0005
TNF					+0,001
Adaptive immunity					
CD4CCR4				-0,02	
CD4CD25					
CD4CD62L	+++0,000	---0,02	+0,000		
CD4CXCR3			+0,01		
CD8CCR4			-0,007		+0,01
CD8CD25			-0,03		
CD8CD62L	+++0,005		0,01		
CD8CXCR3	-++0,04				
CD4CD45RA					
CD4CD45RO	-++0,01	+--0,004		-0,001	
CD4CD69		++-0,006			
CD4HLADR				-0,04	
CD8CD45RA					
CD8CD45RO		++-0,008			
CD8CD69					
CD8HLADR		++-0,007	+0,005		
Innate immunity					
INKT					
NK		++-0,000			
NKT		++-0,004		-0,03	
DC		++-0,02			
MDC					
PDC		++-0,04			

Table 1.b Major determinants of investigated parameters in the preterm neonates without chorioamnionitis (n=25). Just p values suggesting a significant impact are presented. 'Blue', 'red' and 'grey' colours indicate antiinflammatory, proinflammatory and unknown effect on inflammation, respectively. + or - indicates the proportional or inverse relationship (at gestational categories and postnatal age, three signs of polarity are given). For abbreviations see text.

	gest. age category	**age (post-natal)**	**cortisol**	**maternal steroid**
Cytokines				
IL-10/IL-6		+++0,02		
IL4/IFNG				-0,02
MIP1		-+-0,01		
MCP1		-+-0,01		
IL-17		--+0.001		
IL-13		-++0,01		
IL-12		---0,006		
IL-10				
IL-8		---0,008		
IL-7		+-+0,03		
IL-6		---0,0002	-0,02	
IL-5		---0,02	-0,03	
IL-4		-++0,01	-0,01	-0,02
IL-2		--+0,002	-0,001	
IL-1B		+++0,000		
IFN-G				+0,04
GM-CSF		-++0,02		
GCSF				
TNF	-++0,005			
Adaptive immunity				
CD4CCR4				
CD4CD25	-++0,01	--+0,01	-0,01	
CD4CD62L		-++0,002		
CD4CXCR3			+0,04	
CD8CCR4				
CD8CD25			-0,03	
CD8CD62L			+0,01	
CD8CXCR3	-++0,003			
CD4CD45RA				
CD4CD45RO				
CD4CD69				
CD4HLADR				
CD8CD45RA				
CD8CD45RO		++-0,002		
CD8CD69				
CD8HLADR				
Innate immunity				
INKT		-+-0,01		
NK		+++0,000		
NKT	-+-0,001	+++0,03		
DC				
MDC		+++0,02		
PDC				

Table 1.c Major determinants of investigated parameters in the preterm neonates with chorioamnionitis (n=47). Just p values suggesting a significant impact are presented. 'Blue', 'red' and 'grey' colours indicate antiinflammatory, proinflammatory and unknown effect on inflammation, respectively. + or - indicates the proportional or inverse relationship (at gestational categories and postnatal age, three signs of polarity are given). For abbreviations see text.

	gest. age category	**age (post-natal)**	**cortisol**	**maternal steroid**
Cytokines				
IL-10/IL-6				
IL4/IFNG				
MIP1				
MCP1				
IL-17				
IL-13				
IL-12				
IL-10				
IL-8				
IL-7				
IL-6				
IL-5				
IL-4				
IL-2				-0,03
IL-1B				
IFN-G				
GM-CSF				
GCSF				
TNF				
Adaptive immunity				
CD4CCR4				
CD4CD25	-++0,03			
CD4CD62L	+++0,001		+0,02	
CD4CXCR3				
CD8CCR4			-0,002	
CD8CD25				
CD8CD62L	+++0,01		+0,04	
CD8CXCR3	--+0,03			
CD4CD45RA				
CD4CD45RO	-++0,03	+--0,01		+0,002
CD4CD69		++-0,01		
CD4HLADR				
CD8CD45RA		---0,03		
CD8CD45RO		++-0,04		
CD8CD69				
CD8HLADR		++-0,01	+0,03	
Innate immunity				
INKT				
NK		++-0,0001		-0,002
NKT		++-0,002		
DC		--+0,001		
MDC				
PDC		++-0,02	0,04	+0,03

Discussion

These results obtained from a cohort of preterm neonates clearly indicate that the investigated elements of adaptive and innate immunity including cytokines and cellular players are influenced by a number of factor inherent with the premature birth.

Not unexpectedly the major factor having a large impact on cytokine response is CA. The level of almost each cytokine measured is skewed toward a pro-inflammatory state.

Of note, in the sub-cohort of neonates without CA the measured cytokines presented a characteristic fluctuation during the first postnatal week. While one cannot tell whether the net effect of this fluctuation is pro- or antiinflammatory, one should emphasize that there is some 'physiologic' kinetics of postnatal cytokine expression in the preterm neonate that is masked in the presence of CA. (In the subpopulation of neonates with CA, therefore, cytokine levels did not present any characteristic kinetics). This 'physiologic' kinetics should be taken into account when any investigation on perinatal inflammation is performed.

In contrast with cytokines CD4 and CD8 cells governing the adaptive immune responses of the neonates are not affected by CA. Instead, the prevalence of some subpopulations of CD4 and CD8 cells correlated with neonatal cortisol levels, and with postnatal and gestational age. We could detect this phenomenon either in neonates with and those without CA. Particularly the impact of neonatal cortisol levels on adaptive immune response may have large clinical significance. During NICU therapy steroids can be administered for the prevention some perinatal complications. This finding may indicate that this therapeutic intervention may have some unwanted or unpredicted side effect on the maturation of adaptive immune system of the neonate.

The major determinant of the prevalence of the investigated cellular elements of innate immunity is postnatal age. This observation indicates that the timing of postnatal blood sampling is essential when the innate immune response of a neonate is analyzed. One should note, however, that the effect of postnatal age on innate immune system is probably not influenced by the gestational age, adrenal function or CA.

A further important observation of our work was that maternal steroid therapy has in fact no major effect the neonate's immune system and immune state. The clinicians should trust that the routine treatment of mothers with two shots of steroid to prevent the IRDS is safe in this regard.

Substudy with gene expression analysis

[Manuscript is under preparation]

In a selected subpopulation we also determined gene expression patterns in CD4 and CD8 cells isolated from cord blood specimens from preterm neonates with CA (n = 15), without CA (n = 8) and healthy term neonates (n = 15). For this purpose a Nimblegen 40K chip platform was used.

The gene alterations observed between th epre-specified subgroups were analyzed by a pathway analyzis.

With this substudy the following results were obtained:

The comparison of CD4 gene expression of healthy controls and that of preterm neonates without CA indicated the different functioning of the following pathways:

Pathway	**p level**
CIRCADIANPATHWAY	0
HSA04710_CIRCADIAN_RHYTHM	0.004

The comparison of CD4 gene expression of healthy controls and that of preterm neonates with CA indicated the different functioning of the following pathways:

Pathway	**p level**
HCMVPATHWAY	0.012
CIRCADIANPATHWAY	0.012
ERK5PATHWAY	0.048
HSA04710_CIRCADIAN_RHYTHM	0.038
PELP1PATHWAY	0.039
ST_IL_13_PATHWAY	0.042

The comparison of CD8 gene expression of healthy controls and that of preterm neonates without CA indicated the different functioning of the following pathways:

Pathway	p level
ARENRF2PATHWAY	0.007
SA_G2_AND_M_PHASES	0.038
PHENYLALANINE_TYROSINE_AND_TRYPTOPHAN_BIOSYNTHESIS	0.04

The comparison of CD8 gene expression of healthy controls and that of preterm neonates with CA indicated the different functioning of the following pathways:

Pathway	p level
MYOSINPATHWAY	0.001
CXCR4PATHWAY	0.034
SA_G2_AND_M_PHASES	0.006
PLCPATHWAY	0.001
HSA00130_UBIQUINONE_BIOSYNTHESIS	0.034
PKCPATHWAY	0.043
TCAPOPTOSISPATHWAY	0.048

The comparison of CD4 gene expression of preterm neonates with and without CA indicated the different functioning of the following pathways:

Pathway	p level
HSA04080_NEUROACTIVE_LIGAND_RECEPTOR_INTERACTION	0.00106045
PEPTIDE_GPCRS	0.0061665
GPCRDB_CLASS_A_RHODOPSIN_LIKE	0.00733753
HSA01430_CELL_COMMUNICATION	0.01406926
ANDROGEN_AND_ESTROGEN_METABOLISM	0.02604167

HSA04742_TASTE_TRANSDUCTION	0.0349076
MONOAMINE_GPCRS	0.04014168
HSA04340_HEDGEHOG_SIGNALING_PATHWAY	0.04192872
ACE2PATHWAY	0.04587156
INFLAMPATHWAY	0.04655493
STEMPATHWAY	0.04964539

The comparison of CD8 gene expression of preterm neonates with and without CA indicated the different functioning of the following pathways:

Pathway	**p level**
HSA03020_RNA_POLYMERASE	0.033
RNA_POLYMERASE	0.042
AGPCRPATHWAY	0.045
EXTRINSICPATHWAY	0.001
HSA00601_GLYCOSPHINGOLIPID_BIOSYNTHESIS_LACTOSERIES	0.009
FIBRINOLYSISPATHWAY	0.011
ERYTHPATHWAY	0.039
HSA00920_SULFUR_METABOLISM	0.038

Discussion

Our gene expression analysis revealed a number of pathways that may be implicated in the altered immune response of preterm neonates with and without chorioamnionitis. While the clinical relevance of our findings is currently uncertain, it may provide a base for further research in the field.

Further studies done with the support of OTKA grant 76316

Series I.

As an extension of our original proposal we also made efforts to characterize the activation of CD4 lymphocytes in the neonatal period. For this purpose we developed a methodological platform for flow cytometry. With this novel tool we had an opportunity to monitor the fluctuation of some intracellular parameters in CD4 cells following an aspecific activatory stimulus.

In a series of experiments we analyzed how calcium response differ between the term neonate and the adult. We also investigated calcium response in CD4 cells taken in preeclampsia, a major risk factor of preterm birth. The subject of our work was to analyse the contribution of cell potassium channels to cellular calcium homeostasis.

With this novel tool we described striking differences between Th1 and Th2 type CD4 cells.

In addition, we tested CD4 lymphocyte response in a number of immune-mediated disorders. These disorders are hallmarked by an imbalance between Th1 and Th2 cells that resemble to some extent observed in the preterm neonate at risk for perinatal complications.

1.1 Method development

Assay Drug Dev Technol. 2012 Feb;10(1):97-104. doi: 10.1089/adt.2011.0368. Epub 2011 Sep 15.

Kinetic measurements using flow cytometry: new methods for monitoring intracellular processes.

Mészáros G, Szalay B, Toldi G, Kaposi A, Vásárhelyi B, Treszl A.

The aim of our work was to establish flow cytometry methods for the characterization of mitochondrial Ca(2+) levels, plasma membrane potential, and superoxide generation and to relate kinetics to that of cytoplasmic Ca(2+) levels during short-term activation of T-lymphocytes. We monitored the change of fluorescence absorbance of sequentially measured Jurkat cells for 12 min. The cells were stained with the fluorescent dyes Fluo3-AM, Rhod2/AM, di-BA-C4-(5), or dihydroethidium and then were stimulated with increasing doses of phytohemagglutinin (PHA) or were treated with rotenone. Double-logistic function was fitted to cytoplasmic Ca(2+) signal and mitochondrial Ca(2+) levels, whereas logistic function was fitted to plasma membrane potential and superoxide levels. The calculated function parameters were area under the curve (AUC), maximum (Max), time to reach maximum (t(max)), slope at the first 50% value of Max (Slope), and ending (End) values, respectively. We found significant dose-response relationship between PHA dose and cytoplasmic Ca(2+) signals (AUC, Max, Slope: $P<0.05$), mitochondrial Ca(2+) levels (AUC and Max: $P<0.05$), and plasma membrane potential (AUC and End values: $P<0.05$). In rotenone-treated cells, superoxide generation increased in a dose-dependent manner ($P<0.05$ for AUC and End values, respectively). The present methodology provides an opportunity for monitoring and characterizing mitochondrial Ca(2+) levels, plasma membrane potential, and superoxide generation in PHA-activated or rotenone-treated Jurkat cells with flow cytometry.

1.2 T-cell activation in the term neonate

Int Immunol. 2010 Sep;22(9):769-74. Epub 2010 Jul 2.

T-lymphocyte calcium influx characteristics and their modulation by Kv1.3 and IKCa1 channel inhibitors in the neonate.

Toldi G, Treszl A, Pongor V, Gyarmati B, Tulassay T, Vásárhelyi B.

Cytokine production in activated T lymphocytes of the term neonate is reduced compared with adults. We aimed to characterize the calcium influx kinetics of activated T lymphocytes in the neonate and to test the functionality and expression of Kv1.3 and IKCa1 lymphocyte potassium channels, important regulators of calcium influx. We isolated lymphocytes from the peripheral blood of nine adults and cord blood of nine term neonates. We measured the calcium influx kinetics with flow cytometry in the T(h)1, T(h)2, CD4 and CD8 T-lymphocyte subsets activated with PHA. We determined the sensitivity of calcium influx to specific inhibitors of the Kv1.3 and IKCa1 channels. We also measured Kv1.3 channel expression using specific antibody. With the exception of the CD4 subset, calcium influx kinetics was decreased upon activation in neonatal T lymphocytes compared with adults. Neonatal T lymphocytes were found to be less sensitive to the specific inhibition of Kv1.3 and IKCa1 channels. The expression of Kv1.3 channels was higher on major T-lymphocyte subsets of newborns except for T(h)1 lymphocytes. Our findings suggest that the characteristics of short-term activation of major neonatal T-lymphocyte subsets are altered compared with adults. The altered function of neonatal lymphocyte potassium channels may contribute to this phenomenon.

1.3 T-cell activation in preeclampsia, a major risk factor for pregnancy

Am J Reprod Immunol. 2011 Feb;65(2):154-63. doi: 10.1111/j.1600-0897.2010.00899.x.

Lymphocyte calcium influx characteristics and their modulation by Kv1.3 and IKCa1 channel inhibitors in healthy pregnancy and preeclampsia.

Toldi G, Stenczer B, Treszl A, Kollár S, Molvarec A, Tulassay T, Rigó J, Vásárhelyi B.

PROBLEM:Calcium handling of T lymphocytes is altered in healthy pregnancy (HP) and preeclampsia (PE) compared to non-pregnant (non-P) women. We compared the activation-elicited calcium influx in T lymphocytes in HP, PE and non-P women and tested its alteration upon inhibition of Kv1.3 and IKCa1 potassium channels.
METHOD OF STUDY: The alteration of calcium influx was measured in major T-lymphocyte subsets of 9 non-P, HP and PE women with flow cytometry with or without treatment of cells with potassium channel inhibitors.
RESULTS: The elicited calcium response was lower in HP compared to non-P. In HP, calcium influx was sensitive to potassium channel inhibition in CD8 and Th1, but not in Th2 cells. In PE, calcium influx and its sensitivity to inhibition were comparable to non-P.
CONCLUSION: There is a characteristic pattern of calcium influx in T lymphocytes and its

sensitivity to potassium channel inhibition in HP that is missing in PE, raising the notion that T-lymphocyte calcium handling may have a role in the characteristic immune status of HP.

1.4 Different T-cell activation patterns in Th1 and Th2 cells

Immunobiology. 2012 Jan;217(1):37-43. Epub 2011 Aug 23.

Human Th1 and Th2 lymphocytes are distinguished by calcium flux regulation during the first 10 min of lymphocyte activation.

Toldi G, Kaposi A, Zsembery Á, Treszl A, Tulassay T, Vásárhelyi B.

Preliminary data suggest different intracellular calcium handling of Th1 and Th2 lymphocytes that may contribute to distinct cytokine production patterns. In this study we explored the contribution of the main mechanisms in charge of the elevation and decrease of cytoplasmic free calcium levels, i.e., the endoplasmic calcium release, the calcium release activated calcium (CRAC) channel, the mitochondrial calcium uniporter (MCU), the sarco/endoplasmic reticulum calcium ATPase (SERCA), and the plasma membrane calcium ATPase (PMCA) during the first 10 min of activation in human Th1 and Th2 lymphocytes applying a kinetic flow cytometry approach. We isolated peripheral blood mononuclear cells from 10 healthy individuals. Cells were stained with CD4, CXCR3 and CCR4 cell surface markers to identify Th1 and Th2 cells, respectively and loaded with Fluo-3/AM calcium sensitive dye. Cells were activated with phytohemagglutinine and alterations of cytoplasmic free calcium levels were monitored for 10 min after specific inhibition of the above mechanisms. Our results revealed delicate differences in calcium flux kinetics of Th1 and Th2 lymphocytes. The lower activity of MCU, and therefore of CRAC channels, along with the higher activity of the SERCA pump account for the notion that Th2 cells go through a lower level of lymphocyte activation compared with Th1 cells upon identical activating stimuli. The observed differences in calcium flux of Th1 and Th2 cells may contribute to different calcium handling kinetics and, hence, to distinct cytokine production patterns by these subsets.

1.5 Lymphocyte activation kinetics in immune-mediated disorders characterized by a skewness of Th1 / Th2 ratio toward a pro-inflammatory status (comparable to that in the preterm neonate)

1.5.1. Sclerosis multiplex

J Neuroimmunol. 2011 Aug 15;237(1-2):80-6. Epub 2011 Jul 20.

Lymphocyte calcium influx kinetics in multiple sclerosis treated without or with interferon β.

Toldi G, Folyovich A, Simon Z, Zsiga K, Kaposi A, Mészáros G, Tulassay T, Vásárhelyi B.

Kv1.3 and IKCa1 potassium channels play an important role in the maintenance of calcium-influx during lymphocyte activation and present a possible target for selective immunomodulation. We investigated the calcium-influx characteristics of Th1, Th2, CD4, CD8 T-lymphocytes isolated from multiple sclerosis patients without or with interferon-beta therapy, and its modulation by Kv1.3 and

IKCa1 channel inhibitors using flow cytometry. Specific immunomodulation of the CD8 subset can be reached through inhibition of Kv1.3 channels in multiple sclerosis patients without interferon-beta. However, this effect is not specific enough concerning all lymphocyte subsets influencing the autoimmune response, since it also affects anti-inflammatory Th2 cells.

1.5.2. Rheumatoid arthritis

Immunobiology. 2012 May 23. [Epub ahead of print]

The effects of Kv1.3 and IKCa1 potassium channel inhibition on calcium influx of human peripheral T lymphocytes in rheumatoid arthritis.

Toldi G, Bajnok A, Dobi D, Kaposi A, Kovács L, Vásárhelyi B, Balog A.

OBJECTIVE: The transient increase of the cytoplasmic free calcium level plays a key role in the process of lymphocyte activation. Kv1.3 and IKCa1 potassium channels are important regulators of the maintenance of calcium influx during lymphocyte activation and present a possible target for selective immunomodulation.
DESIGN:Case-control study.
SUBJECTS AND METHODS: We took peripheral blood samples from 10 healthy individuals and 9 recently diagnosed rheumatoid arthritis (RA) patients receiving no anti-rheumatic treatment. We evaluated calcium influx kinetics following activation in CD4, Th1, Th2 and CD8 cells applying a novel flow cytometry approach. We also assessed the sensitivity of the above subsets to specific inhibition of the Kv1.3 and IKCa1 potassium channels.
RESULTS:The peak of calcium influx in lymphocytes isolated from RA patients is reached more rapidly, indicating that they respond more quickly to stimulation compared to controls. In healthy individuals, the inhibition of the IKCa1 channel decreased calcium influx in Th2 and CD4 cells to a lower extent than in Th1 and CD8 cells. On the contrary, the inhibition of Kv1.3 channels resulted in a larger decrease of calcium entry in Th2 and CD4 than in Th1 and CD8 cells. No difference was detected between Th1 and Th2 or CD4 and CD8 cells in the sensitivity to IKCa1 channel inhibition among lymphocytes of RA patients. However, specific inhibition of the Kv1.3 channel acts differentially on calcium influx kinetics in RA lymphocyte subsets. Th2 and particularly CD8 cells are inhibited more dominantly than Th1 and CD4 cells.
CONCLUSION: The inhibition of Kv1.3 channels does not seem to be specific enough in peripheral RA lymphocytes, since anti-inflammatory Th2 cells are also affected to a noteworthy extent.

1.5.3. Ankylosing spondylitis

Clin Dev Immunol. 2012;2012:808724. Epub 2011 Sep 28.

Adaptive immunity in ankylosing spondylitis: phenotype and functional alterations of T-cells before and during infliximab therapy.

Szalay B, Mészáros G, Cseh Á, Ács L, Deák

Our aim was to assess the phenotype of T-cell subsets in patients with ankylosing spondylitis (AS), a chronic inflammatory rheumatic disease. In addition, we also tested short-term T-cell activation characteristics. Measurements were done in 13 AS patients before and during the intravenous therapy

with anti-TNF agent infliximab (IFX). Flow cytometry was used to determine T-cell subsets in peripheral blood and their intracellular signaling during activation. The prevalence of Th2 and Th17 cells responsible for the regulation of adaptive immunity was higher in AS than in 9 healthy controls. Although IFX therapy improved patients' condition, immune phenotype did not normalize. Cytoplasmic and mitochondrial calcium responses of CD4+ and CD8+ cells to a specific activation were delayed, while NO generation was increased in AS. NO generation normalized sooner upon IFX than calcium response. These results suggest an abnormal immune phenotype with functional disturbances of CD4+ and CD8+ cells in AS.

1.5.4. Type I diabetes

Immunol Lett. 2010 Sep 6;133(1):35-41. Epub 2010 Jul 13.

Lymphocyte activation in type 1 diabetes mellitus: the increased significance of Kv1.3 potassium channels.

Toldi G, Vásárhelyi B, Kaposi A, Mészáros G, Pánczél P, Hosszufalusi N, Tulassay T, Treszl A.

Kv1.3 and IKCa1 potassium channels participate in the maintenance of calcium-influx during lymphocyte activation. Kv1.3 channels have a prominent role in specific T cell subsets, presenting a possible target for selective immunomodulation. We investigated the impact of Kv1.3 and IKCa1 channel inhibitors on calcium-influx characteristics in human T cells in type 1 diabetes mellitus. We isolated lymphocytes from 9 healthy and 9 type 1 diabetic individuals and measured the alteration of calcium-influx with flow cytometry in the Th1, Th2, CD4 and CD8 subsets after treatment of samples with specific channel inhibitors. Our results indicate an increased reactivity of type 1 diabetes lymphocytes, which is correlated to their increased sensitivity to Kv1.3 channel inhibition. However, the contribution of Kv1.3 channels to calcium flux is not exclusive for a specific lymphocyte subset as previous reports suggest, but is characteristic for each subset investigated. Therefore, the proposed inhibition of Kv1.3 channels as a novel therapeutic approach for the treatment of type 1 diabetes mellitus may have a major effect on overall lymphocyte function in this disease.

Further studies done with the support of OTKA grant 76316

Series II.

We developed micromethods that enabled us to assess the immune phenotype in a complex manner from a small volume of blood.

With the use of this technique we performed a series of studies in different immune mediated disorders. The results of some of these are of relevance for the better understanding of the alterations immune system in the neonate and / or pregnancies at risk for premature birth.

2.1. Galectin expression in the term neonate

Biol Blood Marrow Transplant. 2012 Oct;18(10):1608-13. doi: 10.1016/j.bbmt.2012.05.008. Epub 2012 May 18.

Prevalence of intracellular galectin-1-expressing lymphocytes in umbilical cord blood in comparison with adult peripheral blood.

Kollár S, Sándor N, Molvarec A, Stenczer B, Rigó J Jr, Tulassay T, Vásárhelyi B, Toldi G.

Umbilical cord blood (UCB) is a promising alternative for the treatment of hematological malignancies. The lower immune reactivity of UCB lymphocytes is a well-known phenomenon; however, immune tolerance mechanisms are not fully elucidated. Galectin-1 has strong immunosuppressive properties and plays a key role in the regulation of immune reactivity. We aimed to determine the properties of intracellular galectin-1 (Gal-1)-producing cells within CD3, CD4, CD8, regulatory T (Treg), and natural killer (NK) cells in UCB compared to adult peripheral blood (APB). We took peripheral blood samples from 22 healthy adults and cord blood samples from 19 healthy, term neonates. Intracellular Gal-1 expression was determined by flow cytometry in the above subsets. Furthermore, we assessed the prevalence of naive and memory T cells that play a role in the regulation of immune reactivity. We also performed functional analyses to assess the effect of exogenous Gal-1 on the rate of proliferation of T lymphocytes isolated from APB and UCB. The prevalence of intracellular Gal-1-expressing CD3, CD4, CD8, Treg and NK lymphocytes was lower in UCB than in APB. However, their capability to produce Gal-1 reaches the level seen in adults. The prevalence of naive cells was higher, whereas that of central and effector memory T cells was lower in UCB compared with APB. Lower Gal-1-producing cell proportion might be due to the naivety of neonatal lymphocytes, as indicated by the positive correlation detected between the number of CD3 lymphocytes expressing intracellular Gal-1 and the prevalence of memory T cells. The intracellular expression of Gal-1 may be down-regulated in neonatal lymphocytes due to the already reduced immune reactivity of UCB. In contrast with previous findings, our results indicate that the administration of exogenous Gal-1 failed to decrease the rate of proliferation in T lymphocytes isolated from either APB or UCB. This suggests that Gal-1-expressing lymphocytes are unlikely to play a major role in mitigating the immune reactivity of UCB.

2.2. Immune phenotype in preeclampsia

2.2.1 Regulatory T cells

Acta Obstet Gynecol Scand. 2008;87(11):1229-33.

Decreased number of FoxP3+ regulatory T cells in preeclampsia.

Toldi G, Svec P, Vásárhelyi B, Mészáros G, Rigó J, Tulassay T, Treszl A.

Systemic inflammation is characteristic for preeclampsia (PE). A hypothesis for immune dysregulation is that the function of regulatory T cells (CD4(+)FoxP3(+), Tregs) inhibiting the activation of lymphocytes is impaired. We investigated the proportion of Tregs and their cellular network in preeclamptic women. Fifteen preeclamptic and 17 healthy pregnant women were enrolled in the 32nd gestational week (median age 29 (range 22-45) and 32 (range 26-38) years, respectively). PE was diagnosed according to international criteria at a median of 30 gestational weeks (range 21-31). Peripheral blood was taken and blood mononuclear cells were isolated. Flow cytometry was used to determine the proportion of regulatory (CD4+FoxP3+) T cells, lymphoid and myeloid dendritic cells, natural killer and natural killer T cells, naive and memory and activated CD4+ and CD8+cells. The proportion of Tregs and that of naive CD4(+)CD45RA(+) cells was lower in preeclamptic than in control women (p=0.025, p=0.04, respectively). The proportion of other investigated cell types did not differ. Low Treg numbers may support the notion that PE shares similar features to autoimmune disorders. Low Treg numbers are not reflected in the proportion of activated lymphocytes, at least in this stage of pregnancy. This does not exclude, however, the functional alterations of these cell types.

2.2.2 IL-17 cells

Am J Reprod Immunol. 2011 Sep;66(3):223-9. doi: 10.1111/j.1600-0897.2011.00987.x. Epub 2011 Feb 10.

Increased prevalence of IL-17-producing peripheral blood lymphocytes in pre-eclampsia.

Toldi G, Rigó J Jr, Stenczer B, Vásárhelyi B, Molvarec A.

PROBLEM:Systemic inflammation is a dominant component in the pathogenesis of pre-eclampsia. Besides the imbalance of Th1 and Th2 cells, alterations of the prevalence of Th17 and regulatory T cells have also been suggested to contribute to inflammation. We aimed to describe the prevalence of these four CD4 lymphocyte subtypes in pre-eclampsia and normal pregnancy, along with that of IL-17-producing CD8 and NK cells.
METHOD OF STUDY:Twenty pre-eclamptic and 22 normal pregnant women were enrolled in this study. Using flow cytometry, we determined the prevalence of IL-17-producing cells among the CD4, CD8 and NK cell subsets. Furthermore, we measured the prevalence of CD4+ Tregs, and Th1/Th2 cells were characterized using cell surface chemokine receptor markers.
RESULTS: We demonstrated that there is a shift not only in the Th1/Th2 but also in the Th17/Treg balance favouring skewness towards a pro-inflammatory status in pre-eclampsia. The proportion of CD8 and NK cells that express IL-17 was also higher in pre-eclampsia.
CONCLUSION: The prevalence of IL-17-producing CD4, CD8 and NK cells is elevated in pre-eclampsia, indicating that both the innate and adaptive arms of the immune system are involved in the development of the exaggerated maternal systemic inflammation observed in this pregnancy-specific disorder.

2.3. Immune phenotype in pregnancies complicated with asthma

Int Immunol. 2011 Nov;23(11):669-77. Epub 2011 Sep 20.

Peripheral T(h)1/T(h)2/T(h)17/regulatory T-cell balance in asthmatic pregnancy.

Toldi G, Molvarec A, Stenczer B, Müller V, Eszes N, Bohács A, Bikov A, Rigó J Jr, Vásárhelyi B, Losonczy G, Tamási L.

Asthma is a common chronic disease that may complicate pregnancy and a risk factor for complications; however, immunological mechanisms of the bilateral interactions between asthma and pregnancy are not fully understood. Healthy gestation is characterized by a sensitive balance of T(h)1/T(h)2/T(h)17/regulatory T (Treg) cells that may be altered in asthmatic pregnancy. The aim of this study was to describe the prevalence of these cell subsets in asthmatic compared with healthy pregnancy. The prevalence of T(h)1, T(h)2, T(h)17 and Treg lymphocytes was identified by cell surface and intracellular marker staining in blood samples of 24 healthy non-pregnant (HNP), 23 healthy pregnant (HP), 15 asthmatic non-pregnant (ANP) and 15 asthmatic pregnant (AP) women using flow cytometry. The T(h)1/T(h)2 cell ratio was decreased in both HP and ANP compared with HNP women; however, no further decrease was observed in the AP group. The T(h)17/Treg ratio was decreased in HP, but not in AP women, compared with HNP data. Healthy pregnancy increased Treg cell prevalence compared with HNP data (4.64% versus 2.98%; $P < 0.05$), and this pregnancy-induced elevation was absent in AP women (2.52% versus 4.64%; $P < 0.05$). T(h)17 cell prevalence was similar in the HP and HNP groups (2.78% versus 3.17%; $P > 0.05$). Asthma increased T(h)17 prevalence in non-pregnant patients (3.81% versus 3.17%; $P < 0.05$), and this asthma-specific increase of T(h)17 cell prevalence was also observed in AP patients (AP versus HP: 3.44% versus 2.78%; $P < 0.05$). The abnormal asthma-dependent T(h)17 elevation together with blunted Treg increase may play a role in the compromised immune tolerance characterizing asthmatic pregnancy.

2.4 Immune phenotype in disorders with minor relevance to perinatal immunology

2.4.1. Immune phenotype in pediatric migraine

Neurol Sci. 2012 Oct 16. [Epub ahead of print]

Lymphocyte subsets in pediatric migraine.

Cseh A, Farkas KM, Derzbach L, Muller K, Vasarhelyi B, Szalay B, Treszl A, Farkas V.

Aseptic inflammation due to activated immune cells has been implicated in the pathomechanism of migraine. We measured the prevalence of regulatory T cells (Tregs), along with that of CD4(+)/CD8(+) lymphocytes and their Th1/Th2 commitment in pediatric migraine. Children and adolescents suffering from migraine without aura, migraine with aura and hemiplegic migraine ictally ($n = 53$, 27, and 20, respectively), also interictally ($n = 33$) were recruited and compared to 24 healthy children. Our results indicated comparable prevalence of Tregs, CD4(+) and Th1/Th2 committed cells. CD8(+) prevalence was lower, and CD4(+)/CD8(+) ratio was higher in ictal phase irrespective of the subtype of migraine. No association between CD8(+) prevalence and gender, body weight, disease onset and attack duration in migraine subtypes was found. CD8(+) prevalence was normal in patients in interictal phase. These results suggest the absence of major systemic alteration of adaptive immunity in children and adolescents suffering from migraine; however, a transient decrease of CD8(+) prevalence during the ictal phase was detected irrespective of the subtype of migraine.

2.4.2. Immune phenotype in pediatric inflammatory bowel disease

World J Gastroenterol. 2010 Dec 21;16(47):6001-9.

Immune phenotype in children with therapy-naïve remitted and relapsed Crohn's disease.

Cseh A, Vasarhelyi B, Molnar K, Szalay B, Svec P, Treszl A, Dezsofi A, Lakatos PL, Arato A, Tulassay T, Veres G.

AIM:To characterize the prevalence of subpopulations of CD4+ cells along with that of major inhibitor or stimulator cell types in therapy-naïve childhood Crohn's disease (CD) and to test whether abnormalities of immune phenotype are normalized with the improvement of clinical signs and symptoms of disease.
METHODS:We enrolled 26 pediatric patients with CD. 14 therapy-naïve CD children; of those, 10 children remitted on conventional therapy and formed the remission group. We also tested another group of 12 children who relapsed with conventional therapy and were given infliximab; and 15 healthy children who served as controls. The prevalence of Th1 and Th2, naïve and memory, activated and regulatory T cells, along with the members of innate immunity such as natural killer (NK), NK-T, myeloid and plasmocytoid dendritic cells (DCs), monocytes and Toll-like receptor (TLR)-2 and TLR-4 expression were determined in peripheral blood samples.
RESULTS:Children with therapy-naïve CD and those in relapse showed a decrease in Th1 cell prevalence. Simultaneously, an increased prevalence of memory and activated lymphocytes along with that of DCs and monocytes was observed. In addition, the ratio of myeloid /plasmocytoid DCs and the prevalence of TLR-2 or TLR-4 positive DCs and monocytes were also higher in therapy-naïve CD than in controls. The majority of alterations diminished in remitted CD irrespective of whether remission was obtained by conventional or biological therapy.
CONCLUSION:The finding that immune phenotype is normalized in remission suggests a link between immune phenotype and disease activity in childhood CD. Our observations support the involvement of members of the adaptive and innate immune systems in childhood CD.

2.4.3. Immune modulatory effects of doxazosin

J Int Med Res. 2009 Nov-Dec;37(6):1982-7.

Effect of 3 months of doxazosin therapy on T-cell subsets in type 2 diabetic patients.

Mácsai E, Cseh A, Budai G, Mészáros G, Vásárhelyi B, Fischer K, Szabó A, Treszl A.

Doxazosin, an alpha(1)-adrenergic receptor inhibitor, is commonly administered to patients with type 2 diabetes, hypertension and nephropathy. The impact of 3 months' doxazosin therapy on the prevalence of activated and regulatory T lymphocytes was analysed in this pilot study of men with type 2 diabetes (n = 10) who received doxazosin 4 mg/day in addition to their ongoing therapy. The prevalence of CD4(+), CD8(+), CD25(+) and CD69(+) cells at baseline and after 3 months of add-on therapy was determined. The prevalence of regulatory T-cells was detected by two different approaches: forkhead box P3 (FoxP3) positivity; and the number of CD4(+)CD25(+high) cells. During 3 months of doxazosin therapy, patients' blood pressure, blood glucose control and lipid profiles all significantly improved. Simultaneously, the prevalence of activated T-cells (CD4(+)CD69(+) and CD8(+)CD69(+) cells) decreased, whereas that of regulatory T-cells increased. These results indicate an immunomodulatory action of doxazosin in type 2 diabetic patients.

Further studies done with the support of OTKA grant 76316

Series III.

During the period of the proposal novel inflammatory biomarkers evolved; commercial tests to measure some of them are already available.

We tested the clinical usefulness of soluble urokinase plasminogen activator receptor (suPAR) and hepcidin levels in different settings.

3.1. suPAR

We extensively tested the usefulness of suPAR test to recognize and monitor inflammatory processes in a wide range of disorders.

3.1.1. suPAR in preeclampsia

Clin Chem Lab Med. 2011 Nov;49(11):1873-6. Epub 2011 Jul 4.

Soluble urokinase plasminogen activator receptor (suPAR) levels in healthy pregnancy and preeclampsia.

Toldi G, Bíró E, Szalay B, Stenczer B, Molvarec A, Rigó J, Vásárhelyi B, Bekõ G.

BACKGROUND:
Preeclampsia is characterized by a maternal systemic inflammatory response and the impairment of maternal immune tolerance present in healthy pregnancy. Soluble urokinase plasminogen activator receptor (suPAR) is a biomarker increasingly used for the monitoring of systemic inflammation. We aimed to assess the levels of suPAR and other markers of systemic inflammation in preeclampsia compared to healthy pregnancy.
METHODS:We determined plasma suPAR, IL-6 and high sensitivity C-reactive protein (hs-CRP) levels in plasma samples of 62 healthy pregnant and 41 preeclamptic women in the third trimester of pregnancy.
RESULTS:Plasma suPAR levels were elevated in preeclampsia [3.18 (2.30-4.71) ng/mL vs. 2.02 (1.81-2.40) ng/mL, p=0.0001, median (interquartile range)]. IL-6 and hs-CRP levels were also higher compared with healthy pregnancy [5.99 (2.97-18.12) pg/mL vs. 1.41 (1.00-2.70) pg/mL, p=0.0001 and 6.60 (3.55-15.40) mg/L vs. 3.90 (2.10-7.25) mg/L, p=0.006, respectively, median (interquartile range)]. Linear regression analyses revealed an association between individual plasma suPAR and log IL-6 levels as well as log hs-CRP levels.
CONCLUSIONS: suPAR levels are elevated in preeclampsia and vary in a narrower range compared with IL-6 and hs-CRP. ROC analysis indicated that monitoring of suPAR levels is a suitable tool for the detection of systemic inflammation in pregnancy.

3.1.2. Connecting tissue disorders

3.1.2.1. Rheumatoid arthritis

Biomarkers. 2012 Oct 4. [Epub ahead of print]

Plasma soluble urokinase plasminogen activator receptor (suPAR) levels in systemic lupus erythematosus.

Toldi G, Szalay B, Bekő G, Bocskai M, Deák M, Kovács L, Vásárhelyi B, Balog A.

Objective: Soluble urokinase plasminogen activator receptor (suPAR) is a biomarker of systemic inflammation. We aimed to characterize plasma suPAR levels in SLE patients. Methods: We measured plasma suPAR, C reactive protein (CRP) and erythrocyte sedimentation rate (ESR) in 89 SLE patients and 29 healthy controls. Results: suPAR and ESR values were higher in SLE than in controls, while CRP levels were comparable. ROC analysis of suPAR levels indicated a cut-off value of 5.70 ng/mL to distinguish patients with high disease activity (SLEDAI >8). Conclusion: suPAR might be an objective marker for identifying SLE patients with active disease.

3.1.2.2. Bechterew's disease

Joint Bone Spine. 2012 Sep 19. pii: S1297-319X(12)00175-3. doi: 10.1016/j.jbspin.2012.06.023.

Plasma soluble urokinase plasminogen activator receptor (suPAR) levels in ankylosing spondylitis.

Toldi G, Szalay B, Bekő G, Kovács L, Vásárhelyi B, Balog A.

Recent studies demonstrated that soluble urokinase plasminogen activator receptor (suPAR) is a valuable marker in the recognition of an inflammatory response. Ongoing inflammation leads to elevated plasma suPAR levels. We aimed to characterize plasma suPAR levels in ankylosing spondylitis (AS) patients compared to healthy individuals in order to reveal if suPAR could be used as a clinical marker of inflammation in AS. We measured plasma suPAR and C-reactive protein (CRP) levels as well as erythrocyte sedimentation rate (ESR) in 33 AS patients at various stages of disease duration and activity and 29 healthy controls. CRP and ESR values were higher in AS patients than in healthy individuals, while suPAR values were comparable (median [interquartile range]: 2.97 [2.57-3.80] ng/mL vs. 2.80 [2.06-3.42] ng/mL, P>0.05). In AS patients, a correlation was detected between BASDAI scores and CRP as well as ESR values but not suPAR levels (P=0.0005, r=0.57 and P=0.01, r=0.43, respectively). Unlike in many other inflammatory conditions, plasma suPAR levels do not reflect inflammation in AS. To assess the inflammatory status in AS, ESR and particularly CRP values are still more appropriate clinical markers. In line with earlier findings, our results indicate that, unlike suPAR, both of these markers are positively correlated with disease activity in AS.

3.1.2.3. Systemic lupus erythematosus

Clin Chem Lab Med. 2012 Jun 21;0(0):1-6. doi: 10.1515/cclm-2012-0221.

Soluble urokinase plasminogen activator receptor (suPAR) in the assessment of inflammatory activity of rheumatoid arthritis patients in remission.

Toldi G, Bekó G, Kádár G, Mácsai E, Kovács L, Vásárhelyi B, Balog A.

Background: Soluble urokinase plasminogen activator receptor (suPAR) is a biomarker increasingly used for the assessment of systemic inflammation. We aimed to evaluate suPAR for the assessment of inflammatory activity in rheumatoid arthritis (RA) patients in remission.
Methods: In our cross-sectional study we measured plasma suPAR and C-reactive protein (CRP) levels as well as erythrocyte sedimentation rate (ESR) in 120 RA patients at various stages of disease activity and 29 healthy age-matched controls.
Results: suPAR, CRP and ESR values were higher in RA patients compared to healthy individuals. When suPAR levels were analyzed according to DAS28 scores of RA patients, suPAR level in the subgroup with DAS28≤2.6 was lower than in the subgroup with DAS28>2.6, but still higher than in controls [4.45 (3.33-5.56) ng/mL vs. 3.66 (3.10-4.67) ng/mL vs. 2.80 (2.06-3.42) ng/mL, $p<0.0001$, median (interquartile range)]. In contrast, CRP and ESR values were comparable in the subgroup with DAS28≤2.6 and in healthy individuals. We further analyzed the correlation between the number of tender and/or swollen joints and suPAR levels in RA patients in remission. suPAR values were significantly higher in patients with four tender and/or swollen joints than in patients with 2-3 or 0-1 tender and/or swollen joints.
Conclusions: While CRP and ESR values indicate remission of the chronic inflammatory process in RA, suPAR values are still elevated compared to healthy individuals. suPAR might be particularly valuable in the recognition of inflammatory activity in patients who are in remission according to DAS28 scores but have symptoms of tender and/or swollen joints.

3.2. Hepcidin levels

Hepcidin is a recently recognized factor that promptly decreases iron levels in inflammation.

3.2.1. Hepcidin levels in preeclampsia

Clin Chem Lab Med. 2010 Oct;48(10):1423-6. Epub 2010 Jul 13.

Hepcidin concentrations and iron homeostasis in preeclampsia.

Toldi G, Stenczer B, Molvarec A, Takáts Z, Beko G, Rigó J Jr, Vásárhelyi B.

BACKGROUND: Plasma iron is increased in preeclampsia (PE) when compared to healthy pregnant women. This is in contrast to inflammation characteristic for PE. The link between iron homeostasis and inflammation is hepcidin. Our goal was to describe hepcidin concentrations and its association

with iron homeostasis in PE.
METHODS: We obtained peripheral blood samples from 30 preeclamptic [gestational age: 36.5 (24-40) weeks] and 37 healthy pregnant women [gestational age: 36 (28-39) weeks] to determine plasma hepcidin and interleukin-6 (IL-6) concentrations, complete blood cell counts and parameters of iron homeostasis [plasma iron, transferrin and ferritin levels and total iron binding capacity (TIBC)]. Hepcidin was measured using mass spectrophotometry. The Mann-Whitney test was used for statistical analysis.
RESULTS:Plasma hepcidin, IL-6, iron and ferritin concentrations were increased ($p<0.05$ for all), whereas plasma transferrin, TIBC and mean corpuscular hemoglobin concentrations were lower ($p<0.05$ for all) in PE compared to healthy pregnant women. No differences were seen in the other parameters investigated.
CONCLUSIONS: Plasma iron concentrations are increased despite high hepcidin concentrations in PE. This might indicate a resistance to the iron-decreasing action of hepcidin.

3.2.2. Hepcidin levels in the early postpartum period

J Obstet Gynaecol Res. 2011 Nov;37(11):1620-4. doi: 10.1111/j.1447-0756.2011.01586.x. Epub 2011 Jul 6.

Serum maternal hepcidin levels 3 days after delivery are higher compared to those measured at parturition.

Gyarmati B, Szabó E, Szalay B, Czuczy N, Toldi G, Cseh A, Vásárhelyi B, Takáts Z.

AIM: Our aim was to investigate the levels of hepcidin at parturition and 3 days after delivery and to relate hepcidin levels to parameters of iron homeostasis.
MATERIALS AND METHODS: We measured hepcidin levels with mass spectrometry in serum samples of 38 term pregnant women taken just prior to and 3 days after vaginal delivery ($n = 23$) or cesarean section (CS) ($n = 15$). Hepcidin levels were related to iron homeostasis parameters and interleukin (IL)-6 levels. Parameters measured before and after delivery were compared with the Wilcoxon test.
RESULTS: Serum iron levels (median, interquartile range) decreased (14.3, 9.6-21.1 vs. 8.9, 6.8-11.5 μmol/L, $P < 0.01$), while hepcidin levels increased (2.73, 2.2-3.45 vs. 10.62, 6.70-15.89 μg/L, $P < 0.01$) by the third day after parturition compared to those measured before delivery. IL-6 levels were comparable before and after delivery. No direct association between serum hepcidin and iron homeostasis parameters or IL-6 levels was found.
CONCLUSIONS: Factors triggering hepcidin synthesis dominate 3 days after delivery. Studies are needed to assess the contribution of hepcidin to iron homeostasis during the periparturition period.

3.2.2 Hepcidin levels in the perioperative period

Orv Hetil. 2010 Oct 24;151(43):1790-4.

[Increased hepcidin levels three days after gynecological interventions].

[Article in Hungarian]

Gyarmati B, Szabó E, Szalay B, Cseh A, Czuczy N, Toldi G, Vásárhelyi B, Takáts Z.

Hepcidin is an endogenous substance that inhibits iron absorption and plasma iron levels. Due to technical reasons its levels are not routinely assessed and data regarding its clinical relevance are limited. We analyzed the alteration of hepcidin levels following gynecological interventions. Hepcidin levels were determined by mass spectrometry, along with the levels of interleukin-6, the main inductor of hepcidin with ELISA in 17 women undergoing gynecological intervention just prior to and three days after the surgery. The results were related to iron homeostasis parameters. A decrease in serum iron (median, interquartile range) (17.85 [15.25-24.9] versus 10.1 [7.6-15.0] μmol/l, $p<0.01$) and transferrin levels (60.3 [55.93-67.18] versus 53.1 [49.7-60.0], $p< 0.01$) μmol/l, simultaneously with an increase in hepcidin (2.75 [2.24-3.51] versus 8.01 [6.8-9.67] μg/l, $p<0.01$) and interleukin-6 levels (ND = not detected) (ND [ND - 2.2] versus 8.15 [2.31-12.86], $p<0.01$). Conclusion: As with other acute phase proteins postoperative hepcidin levels dramatically increase, simultaneously with other changes in iron homeostasis. These results indicate a possible causative relationship between increased hepcidin and decreased iron levels. In clinical practice, determination of hepcidin levels may be indicated for characterization and, possibly, prediction of postoperative iron homeostasis. However, measurement of hepcidin level in clinical practice is unlikely in the near future due to the lack of available kits for routine clinical laboratories.