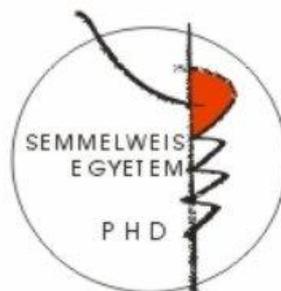# Comparison of bacterial infections and the function of the complement system between patients with and without type 2 diabetes

Outline booklet of the Ph.D. thesis

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1. Introduction

1.1. Diabetes mellitus and infections

Diabetes mellitus is one of the leading factors of morbidity and mortality. The estimated prevalence is 9.5% in the Hungarian population between the age 20-79; the majority of them can be classified into the type 2 diabetic group. 6% of the hospital treatment in relation with infections, and 12% of the mortality of these cases are attributed to diabetes, according to certain estimations. The outcome of the epidemiological studies in relation to this topic are not always coherent, despite its prominent importance almost no Hungarian data are available.

Infection frequency in diabetes mellitus

A relation between tuberculosis prevalence and diabetes was found about 1000 years ago. A strong relation of diabetes with malignant otitis externa, emphysematous pyelonephritis, emphysematous cholecystitis, *Klebsiella pneumoniae* bacterial liver abscess, and melioidosis are known. These diseases, though nowadays are rare, can be observed mostly in patients with diabetes. In the meantime, it is not these types but the infections of the general population that mostly occur in diabetes.

A Canadian study, processing data of 500.000 patients of diabetics and non-diabetics each, found higher relative risks for respiratory (RR: 1.18), urinary (RR: 1.39), and skin and soft tissue (RR: 1.81) infections in a one-year observation period in patients with diabetes compared to non-diabetics. Converging data from Australia, the USA, and Canada showed more frequent hospitalisation of diabetics due to infection in comparison with non-diabetics. The background of the poorer infection risk of patients with diabetes is not well-known enough. Some have doubts regarding this issue emphasising the increased medical attention or the higher rates of recurrent infections among patients with diabetes, which could distort our picture of infection rates.

Infection mortality in diabetes mellitus

Though it is accepted that diabetes could worsen the infection prognosis, the literature on this subject is incomplete, sometimes controversial. Several studies found positive relation between diabetes and infection-related mortality, or length of hospitalisation. Hence, some consider infection as a complication of diabetes. Studies not finding any difference regarding mortality between patients with or without diabetes are also known, moreover other papers reported its opposite: better survival among diabetics having sepsis. Heterogeneity of the criteria of patient inclusion, the types and design of the tests (retrospective or prospective), the numbers and ages of the individuals involved, the severity of disease, the therapeutic protocols varying from country to country (antibiotic and diabetes treatment), and the statistical analyses are suspected to be in the background of the incoherent data.

1.2. Impaired protection against infections in diabetes mellitus

How diabetes could affect infection prevalence and mortality is still a subject of research. Some consider diabetes as an independent risk factor for infectious mortality. Others highlight diabetes complications, glycation, or hyperglycaemia itself. Elevated blood sugar levels were also linked to worse mortality rates of non-diabetics hospitalised due to bacterial infections. This highlights the importance of hyperglycaemia in patients with diabetes as well. On the other hand, the Fremantle Diabetes Study has not found unequivocal association between the higher infection risk of patients with diabetes and their HbA_{1c}-s.

Different pathogen spectrum in diabetes mellitus

Numerous data display different pathogen spectrum, and presence of more aggressive agents among diabetic patients having infections. In urinary tract infections, *Klebsiella*, *Aerococcus*, group B *Streptococcus*, *Proteus* and *Enterococcus* strains occur at a higher relative rate in patients with diabetes compared to non-diabetics. *E. coli* can bind stronger to urethral epithelial cells due to glycation. Some papers report elevated antibiotic

(ofloxacine and cefixime) resistance independent of the type of bacteria, and asymptomatic bacteriuria is more widespread. Regarding respiratory tract infections, *Pneumococcus* was shown to cause bacteraemia more frequently, moreover certain pathogens rather rare in non-diabetics may also be in the background (eg. *Streptococcus agalactiae*). Considering skin and soft tissue infections, diabetic foot has now become an independent clinical entity. Usually they can be described as polymicrobial infections, and beside the most common Gram positive aerobic pathogens Gram negatives, and anaerobics could also occur in large numbers. MRSA and other multiresistant strains have to be considered as well.

The altered immune response in diabetes mellitus

"Barrier" protection mechanisms (intact skin and mucous membranes), cellular and humoral immunity, cytokine and chemokine production, and reactive oxygen species play a role in the protection against pathogen agents of the human body. In diabetes, all these levels of the immunity were shown to have some degree of dysfunction. Higher expression of integrin and endothelial adhesion molecules by neutrophil granulocytes are known to play a role in the enhanced adhesion (glucose on its own is able to stimulate ICAM-1 expression of endothelial cells). Chemotaxis related results are somewhat divided, but it seems it might also be inhibited due to diabetes. Phagocytosis of neutrophils is hampered as a result of the diminished opsonisation. The destruction of the ingested bacteria ("intracellular killing") normally happens with superoxide anions and other free radicals. Defects of this process for many pathogens (*Staphylococcus aureus*, *Streptococcus pneumoniae*, *Candida albicans*) were reported in diabetes, which could parallelly improve with the glycaemic control. Some data showed inhibited degranulation, and lower expression of cytokine and chemokine genes. Regarding the monocytes hampered chemotaxis, phagocytosis, and increased adhesion was described, and their more aggressive intracellular killing mechanisms via the toxic free radicals could lead to cell damage. Decreased lymphocyte transformation response was mostly found in type 1 diabetics. Furthermore, inhibited T-cell proliferation to antigens is also known in patients with diabetes. The hampered phagocytosis secondary to the decreased opsonisation of the neutrophils belongs to the altered humoral immunity as well, but

immunoglobulin (IgG) levels were shown to be lower, and non-enzymatically glycated (which could inhibit the antigen-antibody linkage) in type 1 diabetic patients with poor HbA$_{1c}$. Decreased agglutinin response to typhoid vaccine, and lower antibody response after vaccination of hepatitis A, diphtheria, hepatitis B, and flu were found when compared to patients without diabetes.

So far the complement system came to the forefront mostly in the context of diabetic complications, its possible role in diabetic patients having infections is less known. One of its key elements, the C3, was shown to reach higher concentrations in obesity related hyperinsulinemia (which is present in type 2 diabetes). Moreover, the glycation of C3 is assumed to be linked to the worse opsonisation ability of patients with diabetes, as C3 thus cannot bind to the bacterial surface. Higher levels of C5 and C8 complements were detected in MODY type diabetes. A possible explanation of all these results is the activating effect of the glycated immunoglobulins on the complement system. Based on *in vitro* results, elevated glucose concentration seem to have no implement on the classical and the alternative complement activation pathways, but a diminished activation was computed regarding the mannose-binding lectin- (MBL) related pathway.

1.3. The complement system and its function

The complement system being part of the humoral innate immunity is a cascade system of mostly inactive glycoproteins in the blood and other body fluids. Physiologically it is activated by an appropriate stimulus via regulated proteolysis to explicate its effect. Being part of the innate immunity it can be activated aspecifically, without the presence of T cells or antibodies. According to recent date it plays an important role in the communication with the adaptive immunity, the elimination of pathogens by T and B cells, and the development of the immunological memory.

Its most important functions are lysis (viruses, bacteria, fungus, protozoons, certain cells), opsonisation (marking dangerous antigens by complement proteins, thus phagocytosis is enhanced), developing the inflammatory reaction (recruiting the inflammatory cells, serving their migration and activation), elimination of immune complexes and cell debris,

and linkage to and regulation of the coagulation, the fibrinolytic, and the kinin/kallikrein systems.

The complement system and its activation pathways

The complement system's effective function is important in eliminating dangerous agents, however this process could harm its own organisation as well. Thus, dividing self from non-self has key importance, and PAMPs (Pathogen-Associated Molecular Pattern) and DAMPs (Damage/Danger-Associated Molecular Pattern) play an important role in it. PAMPs are general patterns specific for the microbes (eg. carbohydrate structures on the surface of bacteria, LPS), and DAMPs appear upon stress (eg. DNA released upon necrosis or damage of cells, intracellular proteins). These molecular patterns are recognised by PRMs (Pattern Recognition Molecule) and PRRs (Pattern Recognition Receptor) present on several immune cells and macromolecules, thus initiating the immune response.

The complement system can essentially be activated via three pathways: 1.) Classical path, 2.) Lectin path, and the 3.) Alternative path. Enzymatic cleavage of the C3 complement and thus its activation has key importance, which is triggered by C3-convertases with the same function but different structures, as they are formed in different ways in each complement pathway. From this point the three reaction pathways converge into a fourth, common final (terminal or lytic) pathway, the end of which the TCC (terminal complement complex) is formed. TCC is responsible for the lysis of pathogens.

The classical pathway can be activated by antigen-antibody complexes, CRP, coagulation factor XII, viruses, mycoplasmas, and intracellular components. After the C1 complex binds to antigens, cleavage of C4 then C2 occurs creating the C4b2a, which is the C3-convertase of the classical pathway. Cleavage of the C3 results in the classical pathway's C5-convertase production, which is important in forming the TCC. Molecules formed as "by-products" may behave as anaphylatoxins, and could play a part in the opsonisation. As a by-product of the C4 activation (part of the classical and the lectin

pathways) C4d complement is generated, of which the biological function is unknown, however due to its stability, it gives a possibility to characterise the C4 activation. Hence, C4d has an important role in complement diagnostics.

The C1-esterase inhibitor (C1-inhibitor) is a protein with serine-protease inhibitory function, which explicates its effect on the activated C1 complex and the lectin pathway. Beside this, it has an essential inhibitory role on the kinin-kallikrein, the coagulation (via the activated factor XII), and the fibrinolytic systems.

The lectin pathway is named after the initiators of the process, the lectins: mannose-binding lectin (MBL), ficolin-1, ficolin-2, ficolin-3, collectin-10, and collectin-11. These PRMs are capable of binding the oligosaccharide chains on the surface of the microbes, thus activating the lectin pathway. Lectins are effectors with serine-protease activity, and can activate the MASPs (<u>M</u>BL-<u>A</u>ssociated <u>S</u>erine <u>P</u>rotease). MASPs were name after their capability of associating with MBL, however since then, other lectins were discovered to be able to form complexes with MASPs. Some studies consider the ficolin-3 to be the most potent activator of the lectin pathway. MASP-1 and MASP-2 are activators of the lectin path, and are important in cleaving C4 and C2, thus forming the C3-convertase. The process from this point is similar to the classical pathway. MASP-3 has a regulatory/inactivator role on the lectin path, moreover recently its activator nature on the alternative pathway was discovered. The C1-inhibitor explicates its lectin pathway regulator function via the MASP-2, as previously described.

The alternative pathway's low degree activation normally occurs via the spontaneous hydrolysis of the C3 complement, resulting in formation of the C3b. LPS, "non-self" surfaces (deprived of sialic acid) can intensify this process. Interaction of the C3b with B and D forms the C3-convertase of the alternative pathway. The C3-convertase is able to hydrolyse further C3 molecules generating a self-reinforcing process, which is called amplification. As a result the C5-convertase of the alternative path (C3bBbC3b) is assembled.

C5-convertase enzymes produced via the different pathways fragment C5 into C5a and C5b. C5a is a potent anaphylatoxin. C5b binds C6, C7, C8, and C9, thus forming the TCC. TCC can be generated on the surface of the agent to be lysed: MAC (<u>M</u>embrane

Attack Complex), or it can get isolated from it, thus being able to be detected from the circulation in a soluble form (sC5b-9).

2. Aims

Questions to be answered:

1. Is there a difference between patients with and without type 2 diabetes hospitalised on a general medicine ward due to community-acquired bacterial infections, regarding the following aspects:

   a. The infection localisation,
   b. The pathogen spectrum,
   c. In-hospital and three-months post admission mortalities?

2. Is there a difference in the function of the complement system during the infection between patients with and without type 2 diabetes hospitalised on a general medicine ward due to community-acquired bacterial infections:

   a. Do concentrations of members of the complement pathways, or functional activities of the pathways differ between the two groups?
   b. Is there any relation of the complement system's function with certain clinical parameters or mortality?

3. Methods

3.1. The design of the study

We consecutively involved at least 18-year-old patients hospitalised with clinical diagnosis of community-acquired bacterial infections into our prospective, observational study. Subjects were divided into two groups depending on the presence (T2DM) or absence (ND) of type 2 diabetes mellitus, according to WHO criteria. Based on the statistical power-analysis our aim was to involve 200 patients into each group. Haematological, oncological, and immunological diseases were excluded. Upon patient follow-up we assessed the three-months mortality after the admission. The study protocol was approved by the Scientific and Researchethical Committee (Tudományos és Kutatásetikai Bizottság: TUKEB 396/2013-31584/2013/EKU).

Patient notes and medical documentations were recorded. Basic clinical, infection-related, and hospitalisation-related features were marked of all subjects involved in the study. We assessed the general, the blood sugar- and the glycation-related laboratory results, and the complement parameters.

3.2. Determining the complement and the routine clinical laboratory parameters

Blood sera, EDTA-anticoagulated, and sodium citrate-anticoagulated plasma samples within the first 3 days of hospitalisation were collected from both groups. The blood samples planned to be used to determine the complement parameters were centrifugated (2000x g) in order to divide the cells from the supernatants. Evenly participated sera and plasma samples were then stored until use at -70°C.

Functional determination of the *in vitro* activation potential of the ficolin-3-mediated lectin (F3-LP), the MBL-mediated lectin (MBL-LP), and the alternative (AP) complement pathways were assessed from the blood sera. We selectively activated each pathway, and measured the generated terminal complement complex concentrations. We used commercial ELISA kits according to the manufacturer's instructions (Wieslab, Eurodiagnostica, Malmö, Sweden). Reference ranges based on results of healthy donors:

F3-LP: 25-130%, MBL-LP: 30-130%, AP: 70-125%. The classical pathway activation potential (CP) was determined from blood sera (reference range: CH50 48-103 U/ml) by our laboratory's standardised sheep-erythrocyte haemolytic titration test. It has to be highlighted that these tests assess the blood samples' residual complement activation potential, hence the lower *in vitro* values mean higher *in vivo* activation/consumption.

The ficolin-1 (F1), the ficolin-2 (F2), the ficolin-3 (F3), and the MBL concentrations were measured from blood sera with standardised ELISA technique based on the sandwich-method. ELISA plates were coated with monoclonal antibodies specific for each protein, after incubation of the samples we used biotinylated antibodies. Finally, we applied streptavidin/HRP complexes for detection. Reference ranges: F1: 10–1890 ng/mL, F2: 1.00–12.20 μg/mL, F3: 3–54 μg/mL, MBL: 0–5000 ng/mL.

Complement C3 (reference range: 0.90–1.80 g/L) and C4 (reference range: 0.15–0.55 g/L) levels were measured in serum by turbidimetry (Beckman Coulter, Brea, CA), whereas, radial immunodiffusion was performed to measure the antigenic concentration of C1-inhibitor (reference range: 0.15–0.30 g/L) using polyclonal goat anti-human C1-inhibitor (Quidel, San Diego, CA, USA). Commercial ELISA kits were used to quantify the levels of C4d (reference range: 0.70–6.30 μg/mL) and sC5b- 9, the soluble form of the terminal complement complex (reference range: 110–252 ng/mL); both of these complement activation products were measured from EDTA-plasma (Quidel, San Diego, CA, USA).

C-reactive protein (hsCRP) levels were ascertained by turbidimetry (Beckman Coulter, Brea, CA), other clinical laboratory parameters were measured with Beckman Coulter (Brea, CA) or the Cell-Dyn 3,500 hematology analyzer. Blood glucose levels were determined using the hexokinase assay. Fructosamine was measured with Roche Fructosamine colorimetric test kit (using nitrotetrazolium blue chloride on a Beckman Analyzer AU680, reference range: 205–280 μmol/L). HbA_{1c} concentration was quantified with ion exchange high pressure liquid chromatography (HPLC) method (reference range: 4.0–6.0%). Advanced glycation end product (AGE) levels of both

groups were assessed from the skin by non-invasive autofluorescence technique (AGE Reader mu, DiagnOptics); according to the manufacturer's instructions.

3.3. Definition of sepsis and comorbidities, statistics

Sepsis was defined using the SIRS (Systemic Inflammatory Response Syndrome) criteria: 1. Body temperature >38°C or <36°C, 2. Heart rate >90/min, 3. Respiratory rate >20/min or arterial PCO_2 <32 Hgmm, 4. White blood cell count >12000/mm^3 or <4000/mm^3. Comorbidities were computed based on the points system used for the Charlson Comorbidity Index (CCI). CCI can esteem the 10-year survival in %, based on the cumulative points computed by its points system. This is based on comorbidities of the patients. To be able to compare the comorbidities of the two study groups we calculated the mean CCI points taking into account the following factors: myocardial infarction in history, congestive heart failure, peripheral artery disease, stroke/TIA, dementia, COPD, peptic ulcer, liver failure, hemiplegia, renal failure, and AIDS disease. Points for diabetes were not computed into the T2DM group.

Statistical calculations were carried out using GraphPad Prism 5 (Graphpad Software, USA; www.graphpad.com). Results are presented as medians with 25–75% percentile for continuous, or N; % for categorical variables. To compare variables in two independent groups Mann-Whitney, Fisher's exact test or chi-square tests were used. To analyse associations between variables Spearman correlation was used. All statistical analyses were two-tailed, significance threshold was set at p=0.05.

4. Results
4.1. Clinical results

No difference regarding general clinical or laboratory parameters, age or gender distribution, or CCI points of the comorbidities were found between the 205 T2DM and 202 ND patients. All clinical laboratory parameters in relation to the infection (CRP, PCT, white blood cell count) were higher than their upper reference ranges. Sepsis prevalence

was above 56% for the T2DM patients, and 61% for the ND subjects. No difference regarding any of these parameters were seen between the two groups.

Characterisation of the infections by anatomical localisations

Infections were divided into four groups depending on the infection site: respiratory, urinary tract, skin and soft tissue (SSTI), and other infections. The most common type among diabetic patients was the skin and soft tissue infection (37.1%), and in non-diabetics the respiratory infection (37.1%). Urinary infections followed them in both groups (31.2% vs. 31.7%, respectively). Comparing the infection types between the two patient groups we found higher rates of skin and soft tissue-, and lower frequency of respiratory infections among the T2DM group, compared to ND patients (37.1% vs. 22.8%, p=0.0017 for SSTI, 22.4% vs. 37.1%, p=0.0024 for respiratory). No difference was seen regarding urinary and other infections between the two groups.

Pathogen spectrum and microbiological results

Microbiological culture results taken according to the symptoms of the patients (blood culture, urine culture, wound swab culture) were found to be positive in 55.1% of the diabetic and 48.5% of the non-diabetic patients. Pathogens were categorised according to Gram stain: only Gram positive (Gram+), only Gram negative (Gram-), and cultures containing both Gram+ and Gram- pathogens. Cultures containing both Gram+ and Gram- pathogens were more common in the diabetic group than among non-diabetics (23.1% vs. 9.2% of the positive cultures, p=0.0089, respectively). This difference was attributed mostly to the skin and soft tissue infections (23/76 vs. 5/46, p=0.0149, respectively).

To analyse the urinary tract infections, we divided the pathogens of the positive urinary/blood cultures into the following groups: only *Escherichia coli* (*E. coli)*, only *Klebsiella* strains, other monopathogenic, and polymicrobial. The polymicrobial group contained culture results of more than one uropathogens. We did not observe any difference regarding the positive culture frequencies between the T2DM and the ND groups (73.4 vs. 82.8%, NS, respectively). Increased rates of polymicrobial origin was

observed in the T2DM group when compared to the ND one (34% vs. 15.1%, p=0.0335, respectively).

Mortalities

Neither in-hospital, nor three-months post admission mortality rates differed between the groups (10.7% and 19.0% for the T2DM vs. 9.9% and 20.8% for the ND patients, respectively). The leading cause of death were the skin and soft tissue in the T2DM (23.7%), and the respiratory infections in the ND patients (17.4%).

4.2. Complement results
Comparison of the complement parameters of the study groups

In order to get a reasonably detailed profile on the complement system, concentrations of the recognition molecules of the lectin pathway (ficolin- 1,−2,−3, and MBL), the central complements (C3, C4), the activation product of the classical and lectin pathways (C4d) and the sC5b9 – being able to characterise the overall complement activation – were measured. Moreover, functional activation capacities of the ficolin-3-mediated, and the MBL-mediated lectin pathway (referred to as F3-LP, or MBL-LP, respectively), and the classical (CP) and alternative (AP) complement pathways were determined.

High sC5b9 concentrations seen in both groups indicate a strong complement activation during the infection, however the terminal path activation in T2DM was less elevated than in the ND group (457 vs. 516 ng/mL, p=0.0022). No difference regarding classical pathway (CP) activation was observed between the study groups. On the other hand, *in vitro* values of the F3-LP and AP activation potentials were higher among diabetic patients than in the control subjects (64 vs. 45%, p=0.0354 és 75 vs. 28%, p=0.0013). It is important to be highlighted that these *in vitro* values show the residual complement activation potential, hence they indicate a lower *in vivo* activation/consumption. Weaker *in vivo* F3-LP and AP activation among the diabetics were not present due to the altered concentrations of the recognition molecules or the central complements (F1, F2, F3,

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MBL, C3, C4), as these parameters in the T2DM group were not lower than in the ND patients; as for the C3 it seemed to reach even a higher rate (p=0.0482). We couldn't find any difference in the MBL activation potential (MBL-LP) between the two groups. Importantly, diminished activation of F3-LP in patients with bacterial infections and diabetes was also supported by the significantly lower C4d concentrations, when compared to ND (p=0.0063). Similarly, the significantly higher concentration of C1-inhibitor in the T2DM group compared to ND (p<0.0001) may reflect the impaired activation and consumption of the F3-LP.

To further explore the relationship between consumption of the different pathways (CP, AP, F3-LP) analyses of parallel consumptions were done. Consumption of the pathways were defined according to the lower limit of the reference range (CP: <48 U/mL, AP: <70%, F3-LP: <25%): "lack of consumption" was defined for values equal to or above this threshold. Distribution of complement consumption was polarised in non-diabetic patients: a significant percentage of ND patients fell into subgroups without any consumption (38% for CP/AP, 41% for F3-LP/AP) however, another significant percentage showed consumption of both pathways (45% for CP/AP, and 44% for F3-LP/AP). In contrast, diabetic patients showed different patterns: 42% fell into subgroups without any consumption for CP/AP, and 59% for F3-LP/AP; while only 28% had a consumption of both pathways for CP/AP, and 21% for F3-LP/AP. The T2DM group had less frequent parallel consumptions of F3-LP and AP in comparison with the ND group (p=0.0007).

In addition, when analysing all three pathways together, 48% of ND patients, but only 27% of diabetic patients had parallel activation and consumption of all three pathways: this difference was significant (p=0.037), and was mainly attributable to the diminished activation of F3-LP and AP in diabetes.

F3-LP and AP activation and consumption in different types of infections

Once more, it has to be highlighted, that the increased *in vitro* activation potential observed in the measurement indicate a decreased *in vivo* activation/consumption. The

diminished *in vivo* activation (lack of consumption) of the F3-LP and AP among diabetic patients was most pronounced in case of the UTIs: T2DM subjects had in average 30% higher *in vitro* functional activity of the F3-LP (67% vs. 37%, p=0.0456) and about 51% higher values regarding AP (73% vs. 22%, p=0.0092), when compared to patients without diabetes. Diabetic patients with positive culture results for *E. coli* had diminished F3-LP activation (higher *in vitro* values) compared to those of non-diabetic subjects (70% vs. 33%, p=0.0286). Similar results were observed regarding AP (87% vs. 6%, p=0.0003). However, no difference concerning F3-LP and AP were observed for those with non-E. coli mediated UTIs. In respiratory infections of the diabetic patients, weaker *in vivo* activation was observed (*in vitro* values of 77% vs. 17%, respectively, p=0.0276). No difference regarding other infection sites were computed between the two groups.

Association of complement activation with clinical parameters and mortality

To determine the relation of the complement activation/consumption with the clinical parameters including glycation-related markers, Spearman correlation analysis was done. No associations of F3-LP or AP with blood glucose levels, fructosamine, or HbA_{1c} were found, however, a weak inverse correlation of F3-LP with the long-term glycation marker of AGEs (p=0.0143, r = −0.2765) was observed among T2DM subjects.

Three-months mortality after bacterial infection was similar in the diabetic and non-diabetic groups, however, lack of F3-LP activation/consumption with lack of AP amplification were associated with three-month mortality in the diabetic group (p=0.012 and p=0.025, respectively). Whereas, activation of CP, F3-LP and AP was present in 77, 62, and 76% of ND subjects who died, respectively, the same was not observed for T2DM patients. Although 60% of those diabetic patients who died had CP activation and consumption, this proportion was only 29 and 25% for F3-LP and AP, respectively. There was no difference in the occurrence of complement consumption among the groups with sepsis only.

5. Conclusions

The main conclusions regarding our research focusing on the comparison of the clinical features and the complement activation of patients with and without type 2 diabetes, hospitalised on a general medicine ward due to community-acquired bacterial infections, are as followed:

1. a.) We observed increased prevalence of skin and soft tissue infections among patients with diabetes in comparison with non-diabetics. Its frequency showed to be also higher in comparison with international data. This draws attention to the importance of primary prevention along with its insufficiency in Hungary.

    b.) Regarding the pathogen spectrum, co-presence of Gram positive and Gram negative bacteria were shown to be the most common pathogens in the skin and soft infections of the patients with diabetes mellitus. This showed a difference when compared to the similar infections of non-diabetics. In urinary infections of the diabetic patients, polymicrobial infections were more likely to occur.

    c.) No difference regarding in-hospital or three-months post admission mortalities were found between the diabetic and non-diabetic patients, hospitalised for infection, which serves as supporting evidence for similar international data. Regarding both types of mortalities and both two study groups, Charlson Comorbidity Index points were computed to be increased in patients who died compared to those who survived, moreover they had a higher age in the non-diabetic group.

2. a.) We experienced less increased terminal complement complex formation, and lower *in vivo* ficolin-3-mediated lectin and alternative pathway activation in patients with diabetes compared to non-diabetics. MBL-mediated-lectin and classical complement pathway activations did not differ between the two groups. Parallel consumptions of ficolin-3-mediated lectin, classical, and alternative pathways occurred in a smaller proportion of patients with diabetes in comparison

with those of non-diabetics. These findings suggest that ficolin-3-mediated lectin and alternative pathways play a role in the lower complement activation of type 2 diabetic patients.

b.)   The relation of the glycation parameters with the ficolin-3-mediated lectin and alternative pathways were not clear. The activation/consumption of these two pathways were diminished in those diabetic patients who died three-months after admission.

6. List of publications

Cumulative impact factor (IF): 9.856

6.1. Publications related to the doctoral thesis

Barkai LJ*, Sipter E*, Csuka D, Prohaszka Z, Pilely K, Garred P, Hosszufalusi N. (2019) Decreased Ficolin-3-mediated Complement Lectin Pathway Activation and Alternative Pathway Amplification During Bacterial Infections in Patients With Type 2 Diabetes Mellitus. Front Immunol, 10: 509. **IF: 4.716**
(*These authors contributed equally)

Barkai LJ, Sipter E, Csuka D, Baló T, Nébenführer Zs, Máthé A, Karádi I, Pánczél P, Prohászka Z, Hosszúfalusi N. (2019) Community-acquired bacterial infections among type 2 diabetic and non-diabetic patients hospitalized on a general medical ward: a clinical comparison. [2-es típusú diabéteszesek és nem-cukorbetegek területen szerzett, belgyógyászati osztályos felvételt igénylő bakteriális infekcióinak klinikai összehasonlítása.] Orv Hetil, 160(41): 1623–1632. [Hungarian]. **IF: 0.564**

6.2. Publications unrelated to the doctoral thesis

Kempler M, Baló T, Varga É, **Barkai LJ**, Körner A, Pánczél P, Hosszúfalusi N. (2019) Sometimes nothing is what is seems - Importance of classification in treatment of diabetes through four case reports. [Időnként semmi sem az, aminek látszik - A klasszifikáció jelentősége a cukorbetegség kezelésében négy eset kapcsán.] Diabetol Hungarica, 27(2): 73-78. [Hungarian].

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