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BIOMARKERS – A NEW PERSPECTIVE IN URINARY TRACT INFECTION

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Abstract: *Urinary tract infection continues to be an important cause of morbidity and mortality for adults and children. The early and correct diagnosis is very important considering that upper urinary tract infection can lead to renal scarring, secondary hypertension and, end-stage renal disease. Recent years brought into attention a whole new perspective for the diagnosis, making the biomarkers a very promising field.*

INTRODUCTION

Urinary tract infection – UTI- has a long history, being first documented over 3500 years ago in Egypt and continues to be one of the most frequent infections wide world. The concept refers to any infection that affects any part of the urinary tract. These infections have either bacterial, viral or, fungal etiology but the generally accepted etiology is bacterial when referring to UTI.

It is being estimated that 150 million people experience at least one episode of urinary tract infection, with an incidence four times higher for women compared to men. UTI is responsible for 5% of all episodes of fever in infants.(1,2,3) Fifty percent of the women will have at least a bacterial UTI during their life span.(2) It is a common infection in childhood, occurring in 1.1% of the girls and 1.4% of the boys in the first year of life.(3) Two percent of the boys and four times more girls have at least one episode of UTI by the age of 7.(4)

The early and correct diagnosis is very important considering that upper urinary tract infection can lead to renal scarring, secondary hypertension and end-stage renal disease. 60% of the febrile UTI will result in renal scarring.(5)

Urinary tract infection was a study subject for long, but recent years brought into attention a whole new perspective, the biomarkers' perspective. This field is very promising considering that the „gold standard" for the diagnosis of UTI is urine culture which is a time-consuming investigation. „Urine culture is an imperfect gold standard" having some shortcomings.(6)

The utility of these biomarkers for the diagnosis at the onset of the symptoms is obvious. Finding the right biomarker or the right combination to make an early diagnosis is desirable and would bring many advantages, such as unnecessary antibiotic use or investigations and lower costs in terms of patient health and resources.

Biomarkers in urinary tract infection

To be useful as a biomarker, a molecule has to be organ-specific, secreted promptly by the injured cells, an objective measure of this injury, measurable, reproducible, and sensitive.(7)

Biomarkers used for the urinary tract infection can have two major sources, urine and serum.

Urinary biomarkers

Urine sediment is considered a "good urinary biomarker for early detection of kidney diseases, like acute urinary tract infection".(5) Besides its obvious advantages like accessibility, availability and, low costs, the urine microscopy can offer information about the site of renal injury, inflammation or infection.(5)

Interleukins

IL-6 and IL -8 play an important part in the inflammation of the urinary tract.(3) Studies show that urinary Escherichia coli activates a cytokine response in the monocytes and uroepithelial cells. After E. Coli stimulation the peak concentration was 2 hours for IL-8 and 6 hours for IL-6.(7)

IL-6 is only found in small quantities in the urine of healthy children, but the levels are higher in acute pyelonephritis. The uroepithelial cells secrete cytokines that chemoattract inflammatory cells to the infection site.(8)

IL-6 appears to be correlated with infections with P fimbriae bacterial strains and IL-8 with pyuria.(3,7) IL-8 has higher urinary levels in patients with bacteriuria and it is associated with higher neutrophilia, suggesting its role in attracting neutrophils in the infected urinary tract.(5) Children less than 12 months of age have higher urinary IL-8 levels.(1,5) IL-6 and IL-8 correlate positively in patients with UTI and also correlate with fever, WBC, leucocyturia and CRP.(3,5) Urinary level cannot differentiate pyelonephritis from low UTI in children younger than 2 years of age.(8)

The urinary level was significantly higher in pyelonephritis compared to cystitis or asymptomatic bacteriuria and IL-6 became undetectable when the symptoms disappear and after completion of the antibiotic treatment.(3,7,9) The cytokine level is higher in febrile vs. non-febrile UTI.(3)

Neutrophil gelatinase-associated lipocalin – NGAL- known as lipocalin 2, belongs to the lipocalin protein family which gathers small proteins with very diverse functions and structural roles. Considered initially as transport proteins, their roles are expending: synthesis of prostaglandins and regulation

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of the immune response.(10,11,12)

NGAL, a small positive acute-phase protein, with levels changing during the acute-phase response. NGAL is expressed in many tissues, human neutrophils, monocytes and, macrophages and also in the kidney.(10,13) It is implicated in immune defence against bacterial infection, (13) its levels are upregulated when infection or inflammation is present. The level in the serum can be useful for detecting bacterial infections from viral infections.(5,10,11) It is also an iron-carrier protein.(5)

NGAL is secreted by the injured epithelium of the kidney, is considered one of the earliest and valuable markers of acute kidney injury, one of the most promising immunological biomarkers, a useful marker for the detection of UTI and "has been postulated as a marker".(4,6,12,13,14)

Urine NGAL has high levels in both upper and lower UTI and has significantly lower levels in patients with recurrent UTI. An increase of NGAL has been reported in the early stages of UTI, especially if they are produced by Gram-negative bacterias. This statement is true for both adults and children, mentioning that for children a previous acute kidney injury or chronic kidney disease has to be excluded.(1,5,11,12,13,14)

Beta-2 microglobulin was first discovered in 1964 in the urine of Wilson disease patients or cadmium poisoning.(15) It is a low molecular protein, encoded by a gene in chromosome 15 which can be found on the surface of all nucleated cells. This protein is a component of the cellular immune response. During the inflammatory response that activates lymphocytes, this protein is released into the bloodstream and then passes through glomerular filtration, reabsorption and, excretion. The urinary level is higher if any injury occurs in the proximal tubules.(13,15,16)

The immunological roles are complex and due to interaction with classical and non-classical MHC-1 molecules: mucosal immunity, maternofetal immune tolerance, or tumour surveillance.(15)

In UTI, its levels increase three folds by the third day, making it a valuable but not an early biomarker.(13) The urinary levels are significantly higher in upper tract urinary infection and have normal levels in cystitis, which can make it a valuable biomarker for pyelonephritis. (15,16)

N-acetyl-beta-glucosaminidase –NAG - is a lysosomal enzyme with a molecular weight of 140 kDa. Its hydrolytic function is necessary for the degradation of various parts of the cell. It is found in many tissues but high concentration is present in proximal renal tubular cells. (5,17) Besides acute kidney injury, high levels of NAG can be found in other pathological states, such as UTI, glomerulonephritis, nephrotic syndrome, nephrocalcinosis, urolithiasis, vesicoureteral reflux.(5,18,19,20)

Urinary NAG in children has a circadian variation, with highest levels at the age of 3 and the lowest between six and eight years of age.(5)

In UTI, there is increased urinary excretion of NAG. The levels are higher in pyelonephritis when compared to cystitis, and can be a predictor for UTI for febrile patients.(5,21)

Urinary YKL-40 **–** cartilage glycoprotein-39 or chitinase -3-like-1 belongs to the mammalian chitinase-like protein family. It is expressed in a variety of cells including primary immune cells. It is involved in inflammation, remodeling of the extracellular matrix, angiogenesis, or fibrosis. This biomarker is produced by the injured tissue and is a measure of local inflammation.(22)

The urinary concentration is inversely proportional to the urinary flow rate. The level is significantly higher for patients with UTI than in the control group and in febrile UTI in children. The levels are positively associated with other biomarkers, such as white blood cells, CRP, nitrite, or the pyuria, and negatively with the duration of fever. It appears that the diagnostic value is higher than pyuria or nitrite.(22) Significant higher values of YKL-40 were found in the UTI group compared to febrile children with other infections.(23) The specificity and sensitivity for the diagnosis of febrile UTI are higher than of urinary NGAL. (23)

Serum biomarkers

Immunoglobulins **–** Ig M, Ig A, IgG are significantly higher in patients with UTI than in healthy controls, especially in the reccurent infections. (1)

Procalcitonin – is a peptide with 116 amino acids and a precursor of the hormone calcitonin. It is an indicator of bacterial infection and is considered as a reliable biomarker for the diagnostic of bacterial infections. It expresses the characteristics of a good biomarker, such as specificity, a long half-life, short time of induction and can be detected in serum in 2 hours after the infection.(16)

Procalcitonin is a reliable biomarker that permits to differentiate the pyelonephritis form a lower UTI, in pyelonephritis the levels being significantly higher. This conclusion refers to both adult and children.(7,9,16) It is also a „sensitive indicator for early diagnosis of febrile UTIs in children".(16)

High procalcitonin was proven to be an independent predictor for vesicoureteral reflux, especially in children with a first febrile UTI and also an early predictor of kidney injury in children with UTI. (7,16) It was demonstrated that a high concentration of serum procalcitonin is related to kidney scarring in UTI patients and it is considered an independent risk factor for renal involvement in UTI.(16)

Cytokines

UTI stimulates the release of both local and systemic cytokines and the response is very variable with the severity of the infection.(7)

Interleukins as IL-5, Il-6, IL-8 were extensively studied in relation to UTI and the results show that they are promising and reliable biomarkers for early diagnosis.(24)

As recent studies show, IL-6 and IL-8 were related with age, gender, symptoms, risk factors such as vesico-ureteral reflux, and other biomarkers. They had higher levels in children with febrile UTI compared to the asymptomatic children.(7,9) Children with pyelonephritis have higher levels of IL-6 compared to those who have lower UTI or those in the control group.(9,24)

The sensitivity and the specificity are good, but IL-6 appears to have better sensitivity and specificity than IL-8. (7)

IL-5 has higher levels at the onset of recurrent cystitis and also predicts the development of chronic cystitis in experimental models.(1,25)

Vitamin D

Vitamin D exerts its function on many levels in the organisms, related to the distribution of vitamin D receptor (VDR). The major role is in mineral and bone metabolism but has important immunological functions due to the presence of VDR on immune cells such as monocytes and macrophages.(1,26,27,28)

The immunological roles of vitamin D can be summarized:

- stimulation of macrophage maturation, antimicrobial function and antibacterial peptide expression in both macrophages and monocytes
- induction of autophagy as a macrophage response to Mycobacterium tuberculosis infection
- neutrophilic phagocytic function and motility
- antiviral effects
- immunomodulator by preventing macrophages to release

cytokines too excessively
- suppress inflammation.(1,26,27,28)

Vitamin D deficiency is a risk factor for bacterial infections: respiratory, digestive, and urinary tract infection. Recent studies show that both adults and children with vitamin D deficiency are more prone to infections.(26,27)

In urinary tract infection, in particular, vitamin D induces expression of cathelicidin and β-defensin with bactericide efect on intracellular bacteria. (24,26) Cathelicidin is strongly stimulated by vitamin D. Cathelicidin produced by epithelial cells in the urinary tract exerts a protective role against bacterial adherence. Studies show that low levels of vitamin D are related to low levels of cathelicidin and associated with UTI, while patients with sufficient levels of vitamin D have higher levels of cathelicidin.(27,29)

Urinary tract infection is dependent of vitamin D status.(27,29) Vitamin D and UTI is a subject of recent studies that conclude as follows: vitamin D is a risk factor for infections in general and UTI, in particular, children with pyelonephritis have lower levels of vitamin D in serum.(28)

Vitamin D deficiency in children is independently associated with UTI (29) associated with recurrent UTI (1) and for patients with kidney transplant vitamin D deficiency is an independent risk factor for UTI.(26)

CONCLUSIONS

Urinary biomarkers are very diverse and promising. It is a challenge to find the perfect combination with good specificity and sensitivity to achieve prompt and accurate diagnosis.

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