Quantitative Immunologic Assessments of the Kidney
Inflammatory Infiltrate in Sepsis

LAURIAN LUCIAN FRÂNCU, EDUARD CRAUCIUC, CLAUDIA CRISTINA TÂRNICERU, SCUTARU MONICA, DOINA LUCIA FRÎNCU
Gr.T. Popa University of Medicine and Pharmacy, Iaşi, ROMANIA
e-mail: crauciuc@yahoo.com

Abstract: The infection and inflammation are the most important causes of the disfunction and multiple organic insufficiency in sepsis. In the present work we intended to study the characters of the kidney inflammation in sepsis, also identifying the main cell types by using immunohistochemical methods (CLA – common leukocyte antigen, CD45RO for T lymphocytes, CD20 for B lymphocytes, CD68 for macrophage and the neutrophil elastase for the polymorphonuclear neutrophil) and by quantifying their distribution with the help of an interactive digital programme. These aspects are later correlated with the microorganisms involved and the evolution of the illness/disease. The quantitative microanatomical investigations proved the differential distribution of the inflammatory cells in the kidney and also the different cell densities of the kidney anatomical components. The neutrophiles have the highest index of infiltration in the interstice, and in the glomerule the highest density is in the positive T lymphocytes CD5 and macrophages. In conclusion, the qualitative immunohistochemical study, added to the quantitative computerized study, reveals the major role of the inflammatory cells, like neutrophiles, T lymphocytes and macrophages, in accomplishing a morphologic basis of the kidney affection in sepsis and objectively support the necessity to apply therapeutic measures for the modulation of the inflammation.

Key words: Neutrophiles, T lymphocytes, kidney, macrophages, sepsis.

1 Introduction

The studies made recently in the field of immunology [1],[4],[6],[9] cleared up the functional properties of the inflammatory cells, of the phenotypical changes, and established the role and control of the mediators that are freed by these cells in the kidney tissue. All the research has the role of facilitating the finding of some ways to modulate the kidney inflammation in order to control and limit the severity of the kidney injury, but also to promote the repairing of the injured tissues and to induce tolerance.

The involvement of the kidney in sepsis is proved by the presence of the histopathological lesions especially through the inflammatory aspects met, proving the crucial role of the inflammation in the appearance and progression of the kidney lesions. The kidney takes part with all its structures at the appearance of the inflammatory aspect, the best represented inflammatory infiltrate being at tubulointerstic level.

Many types of cells ensure the response of the host in sepsis, among these being the macrophages, the polymorphonuclear and endotelial cells [1]. The study of macrophages has aroused a new interest lately as a consequence of discovering the role of performing cell in atherosclerosis and organ rejection.

Sepsis is the result of the interaction between the microbial molecules, the cellular derivated mediators (cytokines, derivates of the arahidonic acid, nitric oxide, PAF, lysosomal constituents and vasoactive amines) and plasmatic derived mediators (the complement systems, coagulation-fibrinolisis and kallikrein-kinin) [3],[8].

In the present work we intended to study the characters of the kidney inflammation in sepsis, using immunohistochemical methods in order to identify the main cellular types and quantifying their distribution with the help of an interactive digital programme, because the semiquantitative evaluation with (-), (+), (++) and (+++) is not enough. At the same time, the results obtained are correlated with the microorganisms involved and the evolution of the disease/illness.

2 Material and Methods

For investigating the kidney inflammatory process in sepsis, we performed immunohistochemical studies at the Laboratory of Pathological Anatomy and Mortuary of the Military Hospital Iasi and at the Laboratory of Immunohistochemistry of Polyclinic no. 1, Iaşi.
In order to identify the main cellular components of the kidney inflammatory infiltrate, we used markers for the monocito – macrophagic system, polymorphonuclear neutrophils, markers for immunophenotyping the cells belonging to the lymphocyte line (B lymphocyte, T lymphocyte). In order to show the location and type of the kidney inflammation we used tissue sections that were processed by using the paraffin technique, these samples coming from patients deceased of sepsis. The sections were coloured specifically and examined at the optic microscope, with a lens of 10x, 20x, 40x.


The antigenic cellular components can be distinguished through direct or indirect methods. In our investigation we used the indirect method that uses a cromogenic sublayer which is attached to the secondary antibody, and the main antibody, unmarked, is tied to the secondary one in the antigenic section. This method allows us to see the cellular antigens even if they are in small quantities. The work protocol is the same for all the studied markers, with few differences concerning the times and solutions used in certain phases. We used diaminobenzidine (DAB) as a cromogenic sublayer 3.3” in all the cases, and for this reason the positivation of the studied markers is done when a brown precipitate appears. We followed every type of cell that we examined and we located the place of the inflammation (glomerular, tubulo-interstitial, vascular), and after that we studied how the inflammatory infiltrate is disposed by a successive examining of many microscopic fields.

The qualitative immunohistochemical analysis supervised the positivation of the studied cells, on account of the presence of a brown precipitate, quantified the cells in the inflammatory infiltrate ans classified them into: absent (-), minimum (+), moderate (++), abundant (+++), diffuse or focal. We also wanted to see if, inside the diffuse and focal infiltrate, the inflammatory cells have a tendency of forming aggregates or follicles, especially when a lymphocitary infiltration is present. We tried to establish a connection between the degree of inflammation and the intensity of the tissue lesions met.

The quantitative measurements were done in the Laboratory of Quantitative Microanatomy of “Ion Iancu” Institute of Anatomy from“Gr. T. Popa” U.M.Ph. Iaşi, on the representative microscopic sections. The images were taken from the microscope due to a system of acquiring pictures (video camera connected to a PC), and after that we applied the professional programme PRODIT 5.2. this interactive digital programme allowed us to perform many measurements after choosing the desired aquantitative method from the menu, the results being calculated automatically.

When appreciating the density of a structure that is positive immunohistochemically we used the same slides that were evaluated from the qualitative point of view, for every one of the inflammatory cells, glomerular and interstitial. We quantified the structures that are positive immunohistochemically on 50 consecutive fields, using an ocular 10x, a lens 40x with a numeric aperture of 0.75 and a circular field with the diameter of 450 μm and the surface of 0,159043 mm². The index of density of the selected structure (ID or DI) was calculated automatically and it represents the total number of the care reprezintă numărul total al structurii numărâte pe 10 hpf (hpf = high-power field). In this way we quantified ID - B lymphocytes or ID CD20, ID - T lymphocytes or ID CD45Ro and ID CD5, ID - macrophages or CD68, ID - polymorphonuclear neutrophiles or ID EN, in the end comparing their involvement in constructing the inflammatory infiltrate.

3 Results and Discussions

The inflammatory process that is objectified by leucocitary infiltration varies from moderate to intense. We noticed the evolution of the inflammatory process towards abscess or recent fibrose.

On the kidney glomerule we noticed lesions of acute glomerulonephritis with a serious macrophage and neutrophilic infiltration, in direct correlation with the degree of tissue damage. A varied leukocyte infiltration was present at tubulointerstitial level, and we identified all the components of the leukocyte infiltrate, but T lymphocytes show an intense positive reaction, both peritubulary, and perivascular.

Obvious lesions of tubular and glomerular necrosis were accompanied predominantly by T lymphocyte (CD45RO) and neutrophil infiltration. T lymphocytes presented a diffuse disposition, but also formed lymphocites agglomerations, with a tendency to form nodules.

The inflammatory infiltrate is polimorphous, its nature and intensity being in correlation with the
The aetiologic agent involved. The staphilococcal superantigens cause an intense proliferation and activation of T lymphocytes (CD54RO), with a side effect of extended tubular lesions that may come even to acute tubular necrosis.

B lymphocytes with a positive membrane for CD20 showed a minimal reaction, and were distributed at interstitial level.

The positive macrophages for CD68 show signs of activation and infiltrate all the kidney structures, but the same marker highlights the activation of the tubular epithelial cells induced by the pathogenic agents, especially negative Gram bacillus.

The immunohistochemical techniques showed the presence of the inflammatory infiltrate that represents the morphologic basis of the kidney disease in sepsis, accurately differentiating the component cellular categories and their disposition at renal tissue level, but the evaluations were only qualitative and sometimes semiquantitative. For this reason, we tried to objectify the presence of the cellular categories of the kidney inflammatory infiltrate in sepsis so we used quantitative methods in order to quantify the density of the positive cells for a specific marker, intraglomerular and interstitial. The renal interstice can be seen in the cortical, but it is better represented in the medular and is extended to close to the kidney papillas.

Next we will present in short the results obtained at the computerized evaluation.

The quantification of T lymphocytes after they were identified with CD45 marker shows the following values of the density index CD45 (on 10 hpf = high-power field), values that are very close in the two topographic areas: glomerular – 10 / 10 hpf and interstitial – 9 / 10 hpf.

The quantification of macrophages after they were identified with CD68 marker shows the following values of the density index CD68 (on 10 hpf = high-power field): glomerular – 13 / 10 hpf and interstitial – 17 / 10 hpf.

The quantitative evaluation of B lymphocytes after they were identified with CD20 marker shows the following values of the density index CD20 (on 10 hpf = high-power field): glomerular – 7 / 10 hpf and interstitial – 9 / 10 hpf. The values are close, the distribution of B lymphocytes in the inflammatory infiltrate in sepsis is balanced in the two topographic areas.

The quantitative evaluation of T lymphocytes after their identification with CD45RO marker reveals the following values of the density index CD45RO (on 10 hpf = high-power field): glomerular – 6 / 10 hpf and interstitial – 25 / 10 hpf. With this marker we found the T lymphocytes that populate mostly the inflammatory interstitial infiltrate of the kidney in sepsis.

The quantitative evaluation of the polymorfonuclear neutrophils after being identified with the marker for neutrophil elastaze, shows the following values of the density index NE (on 10 hpf = high-power field): glomerular – 4 / 10 hpf and interstitial – 34 / 10 hpf. In sepsis, the polymorfonuclear neutrophils have the highest index of density in the interstitial inflammatory infiltrate, whereas at glomerular level the density is very small, and may even be a lot of fields where there are not any positive cells found.
Figure 4. The results of quantifying the density of positive polymorphonuclear neutrophiles for the neutrophil elastase in the two locations in the kidney in sepsis

Table 1. Centralizing table with the densities of the inflammatory cells immunohistochemically highlighted in sepsis.

<table>
<thead>
<tr>
<th>CELLULAR TYPE</th>
<th>MARKER</th>
<th>GLOMERULAR LOCATION</th>
<th>INTERSTITIAL LOCATION</th>
</tr>
</thead>
<tbody>
<tr>
<td>T LYMPHOCYTE</td>
<td>CD 45</td>
<td>10 / 10 hpf</td>
<td>9 / 10 hpf</td>
</tr>
<tr>
<td></td>
<td>CD45RO</td>
<td>6 / 10 hpf</td>
<td>25 / 10 hpf</td>
</tr>
<tr>
<td>B LYMPHOCYTE</td>
<td>CD 20</td>
<td>7 / 10 hpf</td>
<td>9 / 10 hpf</td>
</tr>
<tr>
<td>MACROPHAGE</td>
<td>CD 68</td>
<td>13 / 10 hpf</td>
<td>17 / 10 hpf</td>
</tr>
<tr>
<td>NEUTROPHIL</td>
<td>NEUTROPHIL</td>
<td>4 / 10 hpf</td>
<td>34 / 10 hpf</td>
</tr>
</tbody>
</table>

The quantitative evaluation of T lymphocytes after being identified with CD5 marker shows the following values of the density index CD5 (on 10 hpf = high-power field): glomerular – 18 / 10 hpf and interstitial – 25 / 10 hpf. Unlike CD45RO, this marker reveals the presence of T lymphocytes glomerularly, but they have the highest density index in the inflammatory interstitial infiltrate in sepsis.

The inflammatory infiltrate appeared at the kidney level during the systemic infection represents a differentiated apportionment in the kidney anatomical segments. Some inflammatory cells are met mainly in the interstice, some are distributed in a balanced way in the two topographic areas. Cell density is different (in the interstice the highest cell density is in the neutrophiles, followed by T lymphocytes and macrophages. B lymphocytes have the lowest density, so do the positive cells for the common leukocyte marker.

The quantitative microanatomical investigations proved the differentiated distribution of the inflammatory cells all over the kidney and the different cell densities at the kidney anatomical components level. The renal interstice is the topographic area that is most affected when getting an anatomopathological aspect of the septic kidney, while the glomerule has the most reduced infiltration with cells belonging to the immune system. The neutrophiles have the highest infiltration index in the interstice, and the highest density in the glomerule is obtained by the positive T lymphocytes CD5 and the macrophages.

In the renal interstice, the highest value is that of the index of density of the polymorphonuclear neutrophiles, followed by the index of density of positive T lymphocytes CD5 and positive T lymphocytes CD45RO. In glomerular localization the index of density of the positive T CD5 lymphocytes has its maximum value, followed by the index of density of the positive macrophages CD68. In the renal glomerule we identified the moderate macrophagic infiltration in the lesions of acute glomerulonephritis. They are distributed in a diffuse way in the glomerular part, without any tendency of aggregation, whereas in the juxtaglomerular part we noticed a minimum macrophagic reaction. The positive T CD45 and B CD20 lymphocytes have a balanced distribution in the two locations. The density index for the positive CD 68I macrophages has a value above the average in both locations, objectifying their implication in causing the aspect in sepsis.

Normally, the macrophages are absent in the renal glomerule because the migration of the circulatory monocytes appears only in pathologic situations, thus proving their intervention in experimental glomerulonephrites, acute rejections of renal graft. Their activity varies according to the severity of the disease [5].

The leukocyte infiltration, especially the monocyte-macrophagic one plays an important role in the development of glomerulonephritis [4],[7]. We suppose that there is an interaction between the
activated macrophages and the mesangial cells that leads to the activation of the mesangial cells, followed by a release of cytokines that attract even more macrophages in the glomerule, thus determining the progression of the glomerule lesions. The macrophages’ depletion or the inhibition of macrophage infiltration of the glomerule leads to the healing of glomerule lesions and underlines the crucial role of the macrophages in the progression of glomerulonephritis [6].

Our studies made on the kidney in sepsis proved the existence of a mononuclear inflammatory process, going from moderate to intense, in addition to lymphocytes and other cellular types. The macrophages recognize the microbial products, activate the NF-kB that adjust the genic expression of CD14, TLRs and many cytokines. Almost 100 genes are activated transcriptionally by the microbial products. Then the macrophages secrete many mediators including IL-1, IL-6, IL-8, IL-10, GMCSF, TNF, the factor that activates plachetare and leucotriene, but they do not adjust MHC, ICAM, VCAM and the tissue factor [1]. They do not adjust the respiratory fall by inducing the synthesis of the nitric acid (iNOS).

Recent studies indicated the fact that T lymphocytes play an important role in adjusting the immune function and the clearance of the intracellular pathogens. Still, the contribution of T cells in the response of the host to the polymicrobial sepsis remains unclear [9].

The monocytes and macrophages, the T and B lymphocytes, together with many other cells, produce proinflammatory cytokines (IL-6) as a response to the inflammatory stimuli. They induce, together with IL-4 and IL-5, a variety of immunologic effects, interfering in the umoral immunity, stimulating the synthesis of the antibodies and their plasmatic difference. The increased circulant concentration of IL-6 is correlated with a severe prognosis of the sepsis, but it does not exist a direct relation cause-effect [8].

4 CONCLUSIONS

1. In the glomerular location the density index for the positive T CD5 lymphocytes has the maximum value, being followed by the density index of the positive CD68 macrophages.

2. In the renal interstice, the highest value is that of the density index for polymorfonuclear neutrophiles, followed by the density index of the positive T CD5 lymphocytes and of the positive T CD45RO lymphocytes.

3. The positive T CD45 and B CD20 lymphocytes are distributed in a balanced manner in the two locations. The density index of the positive CD 68 macrophages has a value above the average in both locations, thus objectifying their involvement in getting the aspect in sepsis.

4. For the whole, the indexes of density for the immunologic cells, in a decreasing order, include: positive CD5 lymphocytes, positive neutrophiles for the neutrophile elastaze and the positive CD68 macrophages. The other calculated indexes have much smaller values.

5. The qualitative immunohistochemical study, together with the computerized quantitative one, shows the major role of the inflammatory cells, especially the neutrophiles, T lymphocytes and macrophages, in accomplishing the morphologic basis of the kidney illness in sepsis and objectively support the necessity to apply therapeutic measures that concern the modulation of the kidney inflammation.

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