

## Immune status among Moroccans uremic patients

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**ABSTRACT:** End stage renal disease (ESRD) is characterized by disorders of both the innate and adaptive systems. No study is available on disturbance of immune system in ESRD Moroccans patients under dialysis treatment. The aim of this study is to describe the immune status in these patient groups. Our results show the need for a further study in our patients, who are in a state of chronic inflammation following activation of complement, and immune dysfunction leading to impaired function of cells T, B and NK, accompanied by decreased production of antibodies. Understanding the mechanisms behind the immune dysfunction in ESRD generates a perspective to improve lifestyle and reduce mortality in this group of patients.

**KEYWORDS:** Immune status, uremic patients, antibodies.

### INTRODUCTION

Although the proportion of deaths from infection among dialysis patients has markedly decreased in recent years, going from more than 40% in the early 1970s to about 15% from the 1990s in most centers, infection is still second only to cardiovascular disease as a cause of death in end-stage renal disease (ESRD) patients. This high susceptibility of ESRD patients reflects their state of immunodeficiency [2].

It should be noted that the immune dysfunction in uremia is associated with alterations in the two major branches of the immune system, innate and adaptive immunity [3, 4, 5].

Thus, ESRD-associated inflammation is due to activation of innate immune system, orchestrated by monocytes, macrophages, granulocytes, and cellular constituents of other organs/tissues. This is coupled with immune deficiency that is caused by depletion of naive and central memory T cells and B cells, and impaired phagocytic function of polymorphonuclear leukocytes (PMNs) and monocytes. [1, 3]

Immune dysregulation is present in chronic renal failure patients long before the start of dialysis therapy [6], hemodialysis further enhances activation of immune system via dialysis membrane bioincompatibility [7] and impure dialyzate [8], inducing a chronic inflammation.

## OBJECTIVE

The aim of this work is to describe the immune status among hemodialysis patients in Morocco, attempting to deduce the potential role of the disturbances of the innate and adaptive immune systems as cause for the high mortality in this patient population.

## SUBJECTS AND METHODS

### Patients

Fifty chronic hemodialysis patients (twenty nine women and twenty one men) from Department of Nephrology, University Hospital of Casablanca, who gave informed consent were included in this study. Exclusion criteria were the presence of an acute or chronic inflammatory process, the use of immunosuppressive drugs or evidence of malignancy. Mean age of the patient was  $40,41 \pm 8,31$  years (interval of 18 to 60 years). They had been on dialysis at study entry for an average of  $12,04 \pm 6,12$  years. Two patients died from cardiovascular complications during the course of the study. All patients had been treated three times per week.

**Table 1. Baseline clinical characteristics of the study population**

No. of patients	Age (year) Mean ± SD	Sex Ratio (M/F)	Duration in dialysis (year) Mean ± SD	Primary kidney diseases				
				Nephropathy Glomerulo- nephritis	Ischemic nephropathy	Lupus nephritis	Diabetic nephropathy	Unknown etiology
50	40,41 ± 8,31	0,72	12,04 ± 6,12	15	7	2	1	25

### Blood collection

In all ESRD patients, whole blood was collected after cannulation of the vascular access was completed but before the initiation of dialysis.

### Peripheral blood lymphocyte subtyping

Immunophenotypic analysis of the cells was performed using a FACScan flow cytometer (Becton Dickinson).

The percentages of CD3, CD4, CD8, CD11+ CD18+, CD16 + 56+, CD19, CD25, CD38, CD45RA and CD45RO were measured using monoclonal antibodies in peripheral blood samples. For each sample, data from 10 000 cells were collected and analyzed. Forward and side scatters were used to gate and exclude cellular debris. Data analysis was performed using Flowjo software.

We used the blood count formula to convert percentages of lymphocyte subpopulations by cytometrie in absolute value.

### Complement and immunoglobulin by nephelometric assay

Measurement of complement components (C3 and C4) and antibodies (Immunoglobulins G, A and M) was performed by nephelometric methods, using the MININEPH analyser with the recommended dilutions of sera.

**RESULTS**

**Immunoglobulins:**

The prevalence of HBV among patients in our series was 6% (3 patients), whereas it is 46% for HCV (23 individuals). One patient showed a co-infection with both viruses.

All these patients have already been vaccinated against HBV, but only 16 patients (32%) who responded by synthesizing anti-HBV antibodies.

Normal values of immunoglobulins vary by age. Measurement of serum immunoglobulins has detected anomalies in the levels.

**Table 2. Normal values of Immunoglobulin [9-10] and complement components (g / L) in adults. [11]**

IgG	IgA	IgM	C3	C4
6,6 - 12,8	0,7 - 3,4	0,5 - 2,1	0,89 - 1,87	0,165 – 0,380

We observed high levels of IgG in 46 patients (92%), the remaining 4 patients had normal IgG levels. Our results showed also an excessive production of IgA in 14 persons (28%), however only one patient showed a low IgA level (2%).

A high titer of IgM was found in 1 patient, whereas 3 individuals showed an IgM deficiency (6%). (Table 3)

**Complement:**

High levels of C3 and C4 were detected in 18 (36%) and 23 (46%) patients, respectively.

Our results showed C3 deficiency in 3 patients (6%) and decreased C4 level in 5 patients (10%). (Table 3)

No patient in our study presented an associated anomaly of C3 and C4, except one that has a high rate of C4, associated with a low concentration of C3. He has a combined infection of HBV and HCV.

The only patient in our series to be grafted, with an immediate rejection, had high level in C3 and C4 associated with a high rate of IgG and IgM. She has a positive genotyping to HCV RNA (genotype 1b).

We reported also a case of association of C4 and IgM deficiency in the unique patient in the study who had diabetic nephropathy as the primary renal disease.

**Table 3. Frequency of abnormal levels of immunoglobulin and complement components in our series. (%)**

IgG		IgA		IgM		C3		C4	
High	Low								
92	0	28	2	2	6	36	6	46	10

**Lymphocyte phenotyping:**

The cellular components of the immune system are identified and isolated by flow cytometry. Analysis results of cell counts, compared with the normal values (Table 4), showed some disturbances.

**Table 4. Reference values of lymphocyte subpopulations in adult (in absolute value: cells 10<sup>9</sup>/l). [10, 12]**

Leucocytes	LT CD3	LT CD4	LT CD8	LB CD19	NK CD16/CD56	PMNs
1,4 – 3,3	1 – 2,2	0,53 – 1,3	0,33 – 0,92	0,11 – 0,57	0,07 – 0,48	2-7,5

Exploration of subpopulations lymphocyte was performed in 43 patients. All patients in this series have a normal white cell count. However, 22 of 43 patients (51,16%) have lymphopenia.

Lymphopenia or subset deficiency was defined as a value below the 5th percentile of normal values according to age [13].

It was also noticed that the CD4+/CD8+ ratio showed no changes among all patients, because when T cells decrease, both CD4+ and CD8+ are affected.

Also the number of B and NK cells circulating is decreased. In 43 patients, 20 (46.51%) had a decrease in B cell number, while 12 (27.90%) are deficient in NK.

Only one patient showed neutropenia, moreover, it is associated with lymphopenia and a reduced rate of LB and NK.

We analyzed our results about naïve (CD45RA) and memory (CD45RO) T cells, with data reported by Pasquine Saule [14]. Indeed, in 25 patients, we explored distinct- populations in T lymphocytes, naive and memory. Results are reported in table 5.

**Table 5. Results of number of CD4+ and CD8+ T cells subpopulations (% of patients)**

T cells	% in CD4+ T cells			% in CD8+ T cells		
	Normal	High	Low	Normal	High	Low
Naive	84	0	16	52	16	32
Memory (TCM)	12	12	76	52	48	0

Most patients have a normal percentage of naive TCD4 (84%), while 76% have a deficit in CM TCD4.

The number of patients with Naive and CM TCD8 normal levels is dominant (52%), but 32% have a low percentage of naive TCD8. Also 48% of patients have a high percentage of CM TCD8.

CD11b / CD18 are members of the b2 integrin family; Integrin CD11b/CD18 is expressed by neutrophils and monocytes.

Our patients showed high expression of CD11b (88,3%). Only five individuals had a normal expression, they started hemodialysis not more than year. While mean dialysis duration in our patients is 12,04 years  $\pm$  6,12, and 44 patients (88%) were dialyzed for more than 5 years.

A search activation marker CD25 and CD38 was performed only in 10 patients, and preliminary results are in favor of an increase in expression of these markers on T and B lymphocytes.

## DISCUSSION

Several hypotheses have been put forward to elucidate the mechanisms of immune system dysregulation in dialysis patients [2].

An intact complement system, at least through C1, C2, C4 and C3, is necessary for a normal humoral immune response [15-16]. Our data indicates a lack of C3 or C4 in 5 patients (10%).

Patients with a deficiency of C3 have a markedly increased susceptibility to infection with encapsulated bacteria. Similarly, patients with a deficiency of one of the early components of the classical pathway, C1, C4, C2 or in factors H and I (inhibitors of the alternative pathway which causes excessive consumption of C3) also have an increased susceptibility to infection, rheumatic diseases and manifested membranoproliferative glomerulonephritis. Similarly, nearly 80% of C1 deficient patients or C4 deficient patients have presented with a collagen vascular disease [15-17].

Accumulating evidence from the past 30 years has shown the involvement of complement in hemodialysis-related inflammation. Though huge progress has been made towards the selection and manufacturing of dialysis filters with better biocompatibility, these efforts have proven to be insufficient at completely eliminating complement activation and related inflammation [18].

In our series, 26 patients (52%) have high levels of C3 and / or C4. While only 17 individuals (34%) who have normal levels of C3 and C4.

Thus, patients undergoing maintenance hemodialysis still suffer from poor quality of life and low long-term survival rates, warranting further analysis of the mechanisms induced complement activation, to block the unwelcomed effects resulting from chronically acute inflammation

Indeed, the development of complement therapeutics targeting specific proteins and pathways may benefit from the reduced production costs of small peptides compared to other types of drugs, ultimately leading to the development of practical, cost-effective treatments to improve the lifestyle of those undergoing hemodialysis. Clearly, the attenuation of inflammatory complications related to hemodialysis through the inhibition of complement activity already shows high promise and may result in urgently desired novel treatment options for patients suffering from ESRD [18].

It is well established that patients with chronic renal failure exhibit peripheral blood lymphopenia, which can explain the decreased number of T cells observed in our study [19, 20].

This decline in T cells affects both CD4+ and CD8+ and that explains why CD4+/CD8+ ratio is not significantly decreased in our patients [21, 22]. However, many authors support the argument that CD4+/CD8+ ratio is significantly reduced in uremia [3, 23, 24, 25, 26-27].

In our study, 76% of patients have a deficit in CM TCD4, 16% are a reduced naïve TCD4, and 32% have a low percentage of naïve TCD8.

The relative proportions of TCM and TEM in blood vary in the CD4 and CD8 compartments [28], may explain that 48% of patients have a high percentage of CM TCD8.

The selective reductions of the naïve and CM T cells in the peripheral blood of ESRD patients may be due either to increased apoptosis or accelerated activation and differentiation of T cells into EM T cell subsets [29-31, 32].

The increased T-lymphocytes apoptosis concerns mainly the naïve and the central memory T-lymphocytes, but not the effector memory T-lymphocytes [25].

In ESRD patients, a functional T and B cell impairment may be accompanied by decreased production of antibodies to specific stimuli by B cells [19], or by allo-reactive T cell recognition of processed auto-antigens, leading to production of auto-antibodies [33]. This would be in keeping with increased levels of immunoglobulines production, simultaneous to decreasing antibody responses to hepatitis B vaccines in our HD patients.

However, it has been documented that Ig levels, serum IgG isotypes, and both IgM and IgA production are normal in dialysis patients [4, 25]. Even more a change of IgG subclasses was reported, with elevated levels of IgG3 prior to immunisation [34].

The reasons for these conflicting results in studies of immunoglobulins in uraemia are unclear, but may result from defective production of antibodies of individual subclasses in response to certain types of antigen but not others [34].

Indeed, the serological response to strong antigenic stimuli such as CMV is not affected in ESRD patients [35]. However, the response to pneumococcal vaccines that do not require generation of antigen-specific T cells for their efficacy is reduced in these patients compared with that of healthy controls [34, 36].

Increased apoptosis has been confirmed for B-lymphocytes in patients with ESRD [25, 37]. Similar to T cells, progressive loss of renal function is associated with a gradual decline in circulating numbers of B cells, eventually leading to marked B-cell lymphopenia [3, 4, 21, 32, 38-39].

The neutrophil is a critical effector cell in both innate and humoral immunity. However, the capacity for bacterial killing carries with it an implicit capacity for host tissue destruction, as observed in inflammatory and autoimmune disease; accordingly, neutrophil function must be tightly regulated [1, 40, 41].

Natural killer cell function in dialysis patients has been analysed previously [13, 42-47], with conflicting results. Some report increases in NK cell numbers [19-48], or decreases in numbers and cytotoxic activity [13, 42-45, 49-51] or no change [46-47]. In our study, Level of NK cells circulating is decreased in 12 patients (27.90%).

A significant negative correlation was detected between age and NK cell cytotoxic activity, a higher age being related to lower NK cell activity [52].

Migration is an essential anti-infection property of PMNs. It is necessary for a rapid mobilization of these cells to the inflammatory site. Several uremic toxins have the ability to inhibit neutrophil migration in response to classical chemoattractants, thereby reducing the number of effector cells to the inflammatory site and therefore bacterial clearance [53-56]. PMNs migration and adhesion to endothelial cells depends on CD11/CD18 [57].

Complement activation by hemodialysis leads to increased CD11b expression. Pre-treatment with compstatin, which blocks C3 convertase activity and prevents C5a and terminal complement complex production, prevented the increases in CD11b expression [18, 58].

Comparing the results regarding neutrophil functions between different studies is difficult due to the diverse methods and the different conditions of the experiments [59].

The time since the start of dialysis should be taken into account in studies of immune function in dialyzed populations [37]. It can explain the high expression of CD11b showed in our patients (88,3%), as they are in dialysis for  $12,04 \pm 6,12$  years.

Preliminary results of search activation markers CD25 and CD38 are inconclusive, even if they are in favor of an increase in expression of these markers on T and B lymphocytes.

In the perspective of introduce other activation markers such as CD40 in a study with a significant sample.

## CONCLUSION

Disturbances of immunity in HD patients are many and diverse. They are caused by uremia per se, the HD procedure, complications of chronic renal failure, and therapeutic interventions for their treatment. They involve both, the innate and the adaptive systems, generating a complex and still not fully understood immune dysfunction.

Interestingly, the main causes of death in patients with chronic kidney disease (CKD) are cardiovascular and infectious diseases, both being pathologic processes closely linked to immune function.

Therefore, measures aimed at attenuating immune abnormalities in ESRD should be a main research area as this could lead to increased survival and better quality of life in HD patients.

**DISCLOSURES:** None.

**CONFLICT OF INTEREST STATEMENT:** None declared.

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