

Subclinical rejection in renal transplants is associated with low serum mannose-binding lectin levels

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Surveillance biopsies have contributed to the understanding of the natural history of renal allograft lesions. Subclinical rejection, defined as the presence of histological lesions, indistinguishable from acute rejection in stable grafts, is associated with progression of interstitial fibrosis and tubular atrophy. The prevalence of subclinical rejection has decreased as more powerful immunosuppressive treatments have been introduced, suggesting that subclinical rejection represents the degree of control of the alloimmune response. However, non-immune factors such as donor age are also associated with the prevalence of subclinical rejection, suggesting that kidneys from older donors are more susceptible to insult and have a reduced capacity for tissue regeneration. Innate immunity has a crucial role in the modulation of the inflammatory response during infection and tissue damage. Mannose-binding lectin (MBL) is an innate immune protein, the polymorphisms of which are associated with infection, low-grade inflammation, diabetes, and cardiovascular disease. However, the relationship between MBL and disease is complex. For example, low MBL level is associated with higher risk for diabetes, whereas in patients with diabetes, high MBL level is associated with more severe renal damage. In renal transplant patients, low MBL levels are associated with an increased prevalence of infection and diabetes, whereas high MBL levels are associated with shortened graft survival. Although MBL is not clearly associated with prevalence of acute rejection, surveillance biopsy studies have shown that low MBL levels are associated with subclinical rejection in kidney and the heart, suggesting that MBL modulates the injury-repair process of the allograft.

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Protocol biopsy studies have contributed to the understanding of the natural history of renal allograft lesions. These studies have shown that histological damage precedes the appearance of proteinuria or renal functional deterioration in different conditions such as subclinical rejection (SCR), interstitial fibrosis/tubular atrophy (IF/TA), chronic humoral rejection, recurrence of the primary disease, *de novo* glomerulonephritis, or polyoma virus infection. Protocol biopsy studies have shown that early histological lesions constitute an independent predictor of graft outcome. Of all these conditions, SCR has captured the interest of the transplant community for a long period of time.¹

SUBCLINICAL REJECTION AND GRAFT OUTCOME

The term SCR was coined in a study of serial protocol biopsies in 1995 when immunosuppressive treatment consisted of cyclosporine, azathioprine, and prednisone.² In this pioneering study, the prevalence of SCR at different time points during the first year was over 50%. Accordingly, different studies evaluated whether SCR was associated with outcome. In serial protocol biopsy studies, the presence of SCR in a first biopsy was associated with progression of IF/TA, impairment of glomerular adaptation, and progression of glomerulosclerosis in the second one,^{3,4} suggesting that early inflammation favors progression of chronic lesions.

During the 90s, an association between the presence of IF/TA and graft survival was consistently described in protocol biopsy studies. Similarly, in 2005, an association between SCR in 2-week protocol biopsies and graft survival was also documented.⁵ More recently, it has been further described that the association of inflammation and IF/TA in protocol biopsies implies a poorer outcome than IF/TA or SCR alone.^{6–8} Altogether, these observations suggest an association between SCR and poor graft outcome, and raise the question whether treatment of SCR may improve outcome.

PREVALENCE OF SUBCLINICAL REJECTION AND IMMUNOSUPPRESSION

The potential benefit of SCR treatment was explored in a randomized trial in patients receiving cyclosporine, azathioprine, and prednisone. In the study group, patients were biopsied at 1, 2, and 3 months and treated with steroid boluses, whereas the control group was not biopsied and

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accordingly not treated. IF/TA at 6 months and serum creatinine at 2 years were lower in the treatment group, suggesting that treatment of SCR may improve graft outcome.⁹ However, introduction of tacrolimus and mycophenolate reduced the prevalence of SCR to ~10%,^{10,11} showing that SCR can be prevented with more efficient immunosuppression. In a study of serial protocol biopsies, it was observed that reduction of SCR with tacrolimus and mycophenolate was also associated with a lower prevalence of IF/TA at 1 year, in comparison with cyclosporine-based regimens.³ Moreover, in a randomized study comparing a calcineurin-based regimen, either associated with mycophenolate mofetil or associated with sirolimus, the sirolimus groups displayed a lower prevalence of acute rejection and SCR during the first year and a lower prevalence of IF/TA at 5 years.¹² Altogether, these data suggest that SCR prevention with more efficient immunosuppression may improve graft outcome.

RISK FACTORS FOR SUBCLINICAL REJECTION

The close association between immunosuppressive treatment and prevalence of SCR has favored the assumption that it represents, at the histological level, the balance between alloimmune response and efficiency of immunosuppressive treatment. From the epidemiological point of view, it has been described that the degree of sensitization and clinical episodes of acute rejection preceding the protocol biopsy constitute a risk factor for SCR, further reinforcing the notion that SCR represents the intensity of the immune response.¹ However, non-immune factors are also associated with SCR, such as donor age.⁵ Kidneys from older donors are more susceptible to insult and have reduced capacity for tissue regeneration.¹³ Thus, it is tempting to speculate that SCR may not only reflect alloimmune response but also may reflect the inflammatory response associated with tissue injury and repair.

INNATE IMMUNE ALTERATIONS, TISSUE DAMAGE, AND TISSUE REPAIR

Innate immunity constitutes the first line of defense against infection and has a major role in tissue repair.¹⁴ Few highly conserved structures on microorganisms, that is, pathogen-associated molecular patterns, are recognized by pattern recognition receptors that are expressed on effector cells of the innate immune system, such as macrophages, dendritic cells, or B cells. This pattern recognition receptors can be divided into three types: endocytic, signaling, and secreted. Probably, the best-known secreted pattern recognition receptors molecule is mannose-binding lectin (MBL) that activates complement by the lectin pathway and favors inflammation and phagocytosis at the site of infection. Cellular necrosis or apoptosis that follows tissue injury is characterized by the secretion of danger signals known as alarmins, which are sensed by the innate immune system. Recognition of alarmins triggers inflammation and favors phagocytosis of necrotic and apoptotic cells. Once necrotic and apoptotic cells have been cleared, inflammation fades and tissue regeneration leads to injury healing.^{15,16}

As the response to infection and tissue damage is similar, pathogen-associated molecular patterns and alarmins are also termed as danger-associated molecular patterns. Alterations of the innate immune response are associated with inefficient tissue healing, chronic inflammation, and autoimmune disease due to exposure of intracellular antigens and inefficient necrotic and apoptotic cell phagocytosis. All these alterations may also contribute to renal damage after transplantation.

MBL LEVELS AND DISEASE IN GENERAL POPULATION

Innate immune alterations are associated with the prevalence and outcome of different diseases. Different polymorphisms of the *MBL* gene have been described and all of them are associated with decreased MBL levels and impaired MBL function. However, the relationship between MBL and disease is rather complex. Whereas low serum MBL levels are associated with an increased prevalence of infection, autoimmunity, diabetes, and cardiovascular disease, high serum MBL levels are associated with a poorer outcome of certain autoimmune diseases.¹⁷ An example is that low serum MBL level is associated with higher risk for diabetes,¹⁸ whereas in patients with diabetes, high serum MBL level is associated with more severe renal damage.¹⁹ Furthermore, in acute tissue injury after renal ischemia reperfusion injury, high serum MBL level is associated with more severe functional deterioration.²⁰

MBL LEVELS AND RENAL TRANSPLANT OUTCOME

In renal transplantation, an association between MBL polymorphisms or low serum MBL levels and increased susceptibility to cytomegalovirus, bacterial, or fungal infections has been reported in some,^{21–23} but not in all, studies.^{24,25} Despite the higher incidence of infection in low serum MBL patients, no association between MBL levels and mortality has been described.^{26,27} On the contrary, an association between high MBL serum levels and decreased death-censored renal allograft survival has been observed. The incidence of acute rejection was not associated with serum MBL levels, but acute rejection was more often the cause for graft failure in patients with high serum MBL levels, suggesting that high MBL levels may be associated with severe forms of acute rejection.²⁶ Similarly, in kidney-pancreas transplantation, allograft survival was lower in patients with high serum MBL levels.²⁸ In contrast, in heart transplantation, low MBL levels were associated with an increased prevalence of acute rejection and transplant-associated coronary artery disease.²⁹ Taking all these data together, it is difficult to interpret the apparent discrepancies between kidney and heart transplantation. A difference between kidney and heart transplants is that protocol biopsies are usually used to monitor heart histology and, only in few centers, to monitor renal allografts. Furthermore, transplant vasculopathy is actively monitored in the heart by means of coronariography or intravascular ultrasounds. Until now, no studies have analyzed the association between histological

Table 1 | Induction and maintenance immunosuppression in patients with low or high MBL levels

	Low MBL	High MBL	P-value
Induction			
None	1	4	ns
Anti-IL2 antibodies	12	29	
Thymoglobulin	5	9	
Maintenance immunosuppression			
CNI+MMF+P	5	14	ns
mTORi+MMF+P	8	12	
CNI+mTORi+P	3	8	
Belatacept+MMF+P	2	8	

Abbreviations: Anti-IL2, anti-interleukin 2; CNI, calcineurin inhibitors; MBL, mannose-binding lectin; MMF, mycophenolate mofetil; mTORi, mammalian target of rapamycin inhibitors; ns, not significant; P, prednisone.

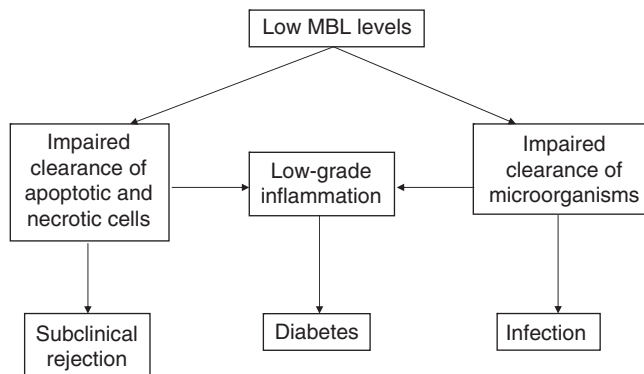


Figure 1 | Relationship between low serum MBL levels and clinical events after renal transplantation. MBL, mannose-binding lectin.

damage in protocol renal allograft biopsies and serum MBL levels.

In a recent study, we measured serum MBL levels after transplantation in a cohort of consecutive renal transplants and classified MBL as low or high according to tertile distribution. The first tertile was considered as the low MBL group and the two higher tertiles were considered as the high MBL group. Despite the fact that donor and recipient characteristics were similar between groups, and that the prevalence of delayed graft function or acute rejection was not different between groups, we observed that patients with low MBL levels displayed more severe low-grade inflammation before transplantation as measured by serum levels of soluble tumor necrosis factor receptor 2, suffered a higher incidence of bacterial or fungal infections, and showed an increased prevalence of new-onset diabetes after transplantation.²³

In the above-mentioned study, a 3-month surveillance biopsy was done in patients with a stable serum creatinine <200 µmol/l and without significant proteinuria (<1 g/day) after obtaining informed consent. There were 60 out of 125 recruited patients with an adequate surveillance biopsy. Histological examination of these biopsies according to the

Banff criteria led to the diagnosis of SCR in 7 of 18 (38.9%) low MBL patients and in 3 of 42 (7.1%) high MBL patients ($P = 0.0054$). Induction and maintenance immunosuppression was not different in patients with low and high MBL levels (Table 1). This observation suggests that innate immune alterations such as MBL deficiency may contribute to the degree of inflammation of the renal allograft (Figure 1). Thus, it is tempting to speculate that patients with low MBL levels may have an impaired capacity for tissue repair leading to more severe and prolonged inflammation.

CONCLUSION

Innate immune alterations modulate different comorbidities in renal transplant patients and may influence graft outcome. Despite this association, mechanisms linking innate immunity and graft damage have not been clearly elucidated. In one hand, it has been suggested that patients with high MBL levels may suffer from more severe episodes of rejection, whereas in other studies low MBL levels have been associated with a higher prevalence of subclinical or clinical rejection. Thus, the relationship between graft outcome and innate immune alterations deserves further studies.

DISCLOSURE

All the authors declared no competing interests.

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