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REVIEW

The time dependency of renal allograft histology

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SUMMARY

Much of the complexity of the histological appearance of kidney transplant biopsies depends on the time at which the biopsies are obtained. It is well established that many elementary histological lesions and diagnoses have a time-dependent occurrence. While some "active" inflammatory lesions are noted primarily early after transplantation, other lesions are "chronic" and accumulate over time post-transplant, sometimes closely related to the prior active inflammatory lesions. With time after transplantation, the complexity of histology increases, by the co-occurrence of chronic damage and specific diseases. This leads to difficulties in clinical interpretation of the histological picture. We discuss the time-dependent prevalence of active and chronic lesions in kidney allograft biopsies and their associations with outcome. We also elaborate on the importance of time post-transplant in the interpretation of complex histological lesions or mixed diagnoses and illustrate that further research is necessary to evaluate whether time post-transplant is important for prognostication of graft injury processes. Adding a multidimensional prognostic layer to the current diagnostic Banff classification, including graft functional characteristics and time after transplantation, could become an interesting aid in the interpretation of complex histological lesions and mixed diagnoses, and in therapeutic decision-making.

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Introduction

Kidney allograft failure remains an important problem on the long term after transplantation, despite marked improvements in short-term graft outcome, by better immunosuppression and prevention of rejection. Chronic histological damage and specific diagnoses have an additive and independent effect on graft failure [1,2]. Although specific diagnoses contribute importantly to graft failure, the histology of late biopsies is very often complex with co-occurrence of extensive chronic damage with specific disease processes, which renders clinical interpretation of late kidney allograft biopsies difficult.

Much of the complexity of the histological appearance of kidney transplant biopsies depends on the time at which the biopsies are obtained. It is very well established that many lesions have a time-dependent occurrence. While some "active" (inflammatory) lesions are noted primarily (but not exclusively) early after transplantation, other lesions are "chronic" and accumulate over time post-transplant [3]. However, the exact time course, whether lesions are transient, reversible or persistent, and which early active lesions lead to which chronic lesions, remains largely unclear.

In this narrative review, we focus on the time-dependency of histopathologic lesions and diagnoses in

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kidney transplant biopsies, the relation and co-occurrence of specific diagnoses and chronic damage, and the prognostic importance of these lesions and diagnoses, and of time itself on the risk for graft failure. Furthermore, we evaluate which lesions and diseases are transient or progressive. Finally, we provide a conceptual framework for identifying surrogate marker candidates for graft outcome, taking into account the time-dependent aspects of graft histology.

Time-dependency of lesions and diagnoses

Active lesions

Early after transplantation, lesions are often more specific and easier to interpret, compared to late histology where the co-occurrence of specific disease processes and chronic damage complicates the histological interpretation (Fig. 1). In the first weeks after transplantation, acute tubular necrosis, antibody-mediated rejection (ABMR), and T-cell-mediated rejection (TCMR) are the most likely diseases to occur, sometimes simultaneously [2,4]. TCMR and ABMR have very different phenotypes and kinetics, and the chronic damage induced by rejection is dependent on the type, persistence, timing, and severity of these episodes of rejection.

An important factor in the prevalence of lesions and diagnoses is the difference between protocol [3,5] and indication biopsy studies [4,6]. Studies reporting on the histology of indication biopsies inherently overestimate the prevalence of lesions and diseases and—per definition—miss subclinical damage. On the other hand, centers reporting on the prevalence of lesions and diseases in patient cohorts followed with protocol biopsies will have higher prevalence than centers only performing indication biopsies if also patients that did not undergo post-transplant biopsies are included. Subclinical lesions and diagnoses will be reported in such protocol biopsy studies but are clearly missed in the centers that only perform indication biopsies. Therefore, only protocol

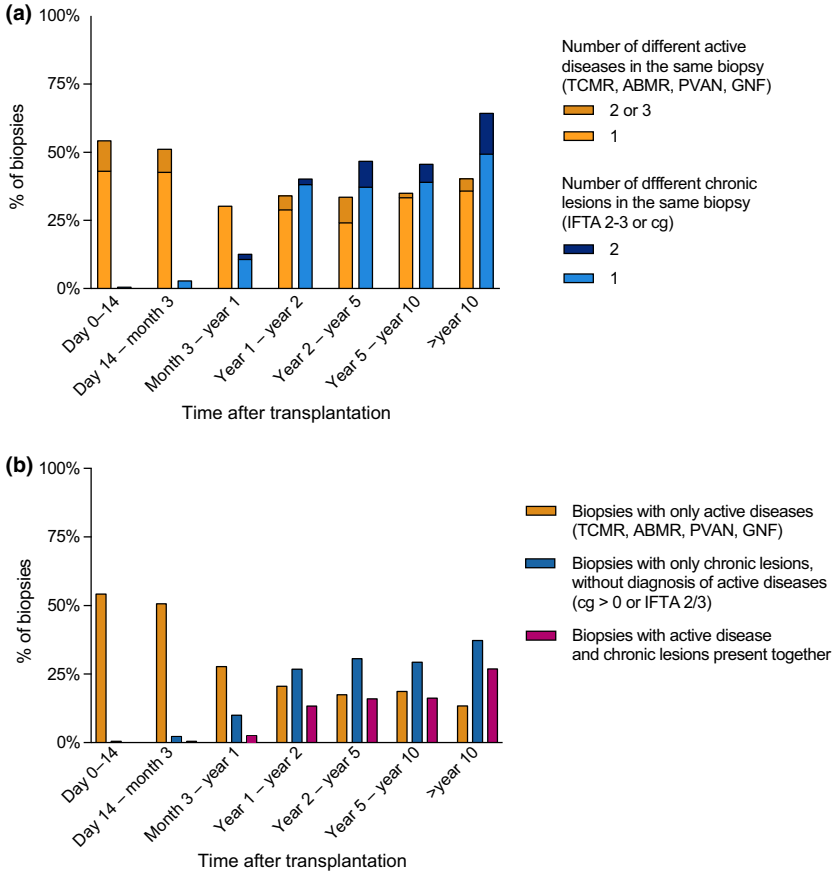

Figure 1 Time-dependent prevalence of histological diagnoses of 1365 indication biopsies described previously [4]. (a) Prevalence of active and/or chronic histological lesions over time. (b) Prevalence of active diseases or chronic lesions only, or overlap of active disease and chronic lesions over time. TCMR, T-cell-mediated rejection; ABMR, antibody-mediated rejection; PVAN, polyomavirus-associated nephropathy; GNF, glomerulonephritis; IFTA, interstitial fibrosis and tubular atrophy; Cg, transplant glomerulopathy [4].

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biopsy studies provide a good view on the time dependency of lesions and diseases, although selection bias and overrepresentation of low-risk patients (who did not lose their graft earlier) impact the data. Very few centers perform protocol biopsies later than 2 years after transplantation, which implies that our knowledge of the evolution of graft histology beyond that point is very limited.

Since the first issue of the Banff classification, the term "acute" is used as adjective with rejection, both for TCMR and ABMR [7,8]. "Acute" was introduced as indicator of disease activity rather than disease timing, as also biopsies late after transplantation could have "acute" TCMR or "acute/active" ABMR. In the Banff 2017 discussions in Barcelona, it was decided to omit the term "acute/active" ABMR and proceed with "active" ABMR, omitting "acute" from the diagnostic classification (unpublished information). For TCMR, a similar semantic change from "acute" to "active" could be considered in future updates of the Banff classification, given that "acute" could be misinterpreted as an indication of early timing, while it is rather used as an indicator of disease activity in the Banff classification. In this manuscript, which discusses the importance of biopsy timing, we avoid the use of the term "acute" to denominate disease activity, but rather use the term "active", in order to not create confusion between "acute" as indication of "early" and "acute" as indication of "activity".

T-cell-mediated rejection

Different grades of TCMR are defined according to the Banff classification[9], ranging from borderline changes to TCMR grade I, II, and III, depending on the grade of tubulitis ("t"), interstitial infiltration ("i"), and intimal arteritis ("v"). The histology of TCMR is nonspecific. These histological lesions do not only represent rejection but can also be seen in ischemia-reperfusion injury or other causes of interstitial nephritis.

Tubulitis and interstitial inflammation are transient lesions [3], which appear predominantly early after transplantation, although these lesions also can be observed in late biopsies (Fig. 2) [3,4]. The decrease in TCMR prevalence in late biopsies is potentially explained by the phenomenon of T-cell exhaustion, which is hypothesized as being a partial adaptive T-cell tolerance over time that depletes their ability to generate TCMR against the graft [10–12]. Persistence of (subclinical) TCMR for more than 1 year, sometimes called "true chronic rejection", is uncommon (only 5.8%) [3].

T-cell-mediated rejection often presents only subclinically [3,13]. Subclinical rejection in protocol biopsies is by definition diagnosed without any evident graft functional deterioration at the time of the biopsy. As subclinical rejection cannot be clinically detected, it can persist unnoticed for quite some time, leaving substantial damage and potentially contributing to chronic and irreversible damage. Many studies indeed reported that subclinical TCMR associates with increased Interstitial fibrosis and tubular atrophy (IFTA) in subsequent protocol biopsies [3,5,14–17]. In a previous study, we found that even inflammation that is quantitatively below the diagnostic threshold of TCMR can be involved in progressive renal allograft damage [18]. In addition, patients with clinical but also subclinical TCMR may develop *de novo* donor-specific HLA antibodies (DSA) and progress to transplant glomerulopathy [13,19,20].

By this, (subclinical) TCMR plays a role in late graft failure. TCMR episodes followed by abnormal histology resulted in reduced graft survival, while TCMR that did not lead to chronic injury was less deleterious [21–27]. In contrast to this, a recent and large study reported that subclinical TCMR at 1 year after transplantation was not associated with an impaired graft survival over 8 years post-transplantation (88% vs. 90% with no rejection) [13]. The reason for this apparent discrepancy is unclear, but could be related to selection bias in the 1-year protocol biopsies, insufficiently long follow-up time or statistical power issues.

The clear association between tubulitis, interstitial inflammation, and concomitant or subsequent IFTA leads to studies that evaluated the impact of inflammation in zones of atrophy, which is classically not taken into account in the Banff classification for diagnosis of TCMR. Until 2015, the Banff criteria for "chronic active" TCMR listed only vascular lesions (arterial intimal fibrosis with mononuclear cell infiltration in fibrosis, formation of neo-intima). At the 2017 Banff meeting in Barcelona, there was growing suggestion that interstitial inflammation in areas of IFTA be used for diagnosis of "chronic active" TCMR (Fig. 2). The deleterious outcome of inflammation in zones of atrophy ("i-IFTA") and IFTA with Banff "i" score > 0 has been established [21,28,29], although its pathogenesis (allo-immune versus nonspecific) remains largely unclear. Further research, especially to determine whether immunosuppression plays a role in the occurrence or disappearance of inflammation in scarred areas, seems necessary before implementing these suggestions in updates of the Banff diagnostic scheme. But if the severity or prevalence of this inflammation in scarred areas relates to the type of

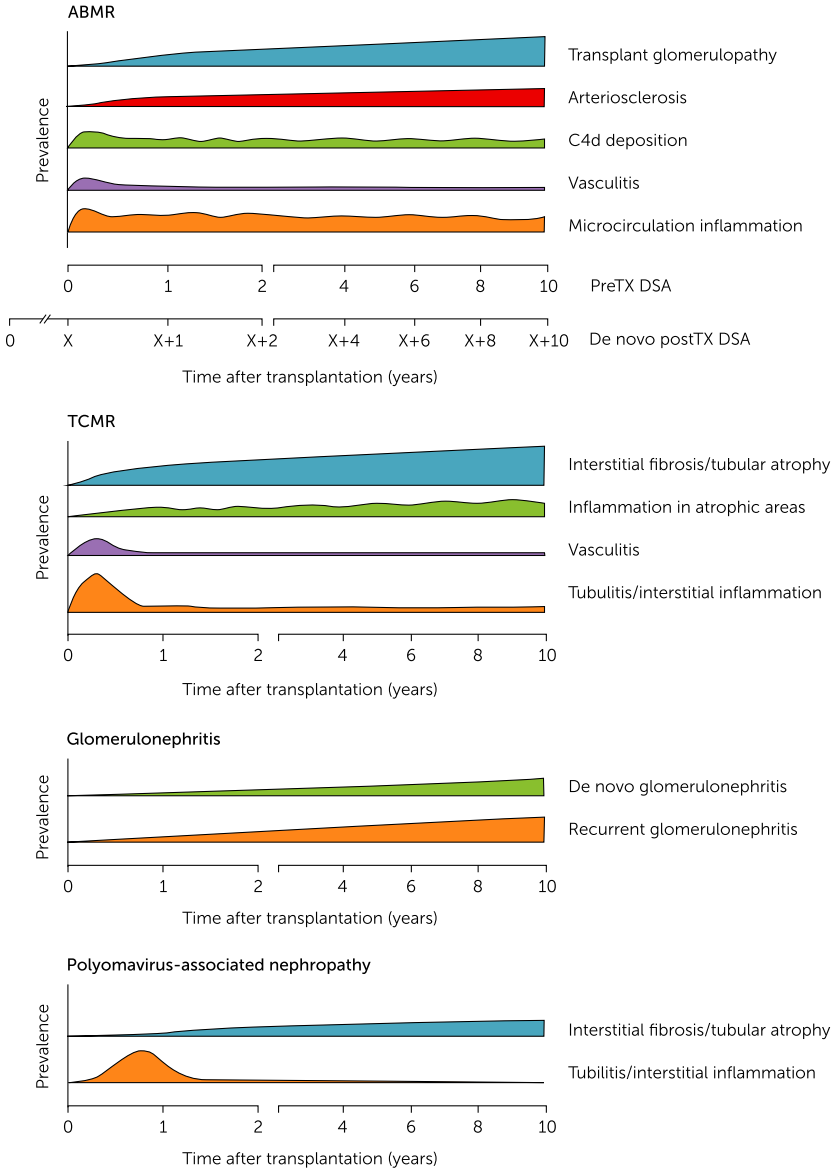

Figure 2 Schematic presentation of the relative prevalence of histological lesions and disease processes, over time after transplantation. These data were not derived from real clinical data but represent a simplified summary of the literature discussed more in detail in the text. These graphs also do not represent the evolution of histology in individual cases, which is highly unpredictable and not necessarily follows the average disease prevalence. Over time, kidneys loose function and graft failure ensues, which is not taken into account in these schematic figures. Vasculitis (intimal arteritis) is a nonspecific lesion that can be associated with both TCMR and ABMR, and also interstitial fibrosis/tubular atrophy and arteriosclerosis are nonspecific. The graphs suggest the relative prevalence of these lesions and diagnoses in relation to the specific cause and to time after transplantation, not the overall prevalence. The *Y*-axis is not defined, given that disease prevalence varies largely in the different cohorts and that there are no data on the exact long-term prevalence of specific lesions and diagnoses. ABMR, antibody-mediated rejection; TCMR, T-cell-mediated rejection; PVAN, polyomavirus-associated nephropathy; *X* = time of occurrence of *de novo* post-transplant donor-specific HLA antibodies.

immunosuppression or decreases with antirejection therapy, inclusion in the Banff classification as an underrecognized pattern of TCMR will be important.

Antibody-mediated rejection

While TCMR is observed mainly early after transplantation and can evolve into developing IFTA and inflamed IFTA, the presentation of ABMR is very different (Fig. 2). The histology of ABMR is characterized by peritubular capillaritis ("ptc"), glomerulitis ("g"), transplant glomerulopathy ("cg"), and C4d deposition in peritubular capillaries. This histological presentation of ABMR is more specific than that of TCMR. Combined presence of these lesions, in the presence of donor-specific HLA antibodies (DSA), is considered to be diagnostic of

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ABMR [8]. Nevertheless, it has to be emphasized that peritubular capillaritis can be seen in the absence of antibodies in the context of TCMR, albeit not considered for diagnosis of TCMR. Vasculitis ("v") lesions can be present in ABMR, but also in TCMR, and cannot be used to discriminate between these two rejection types.

Despite the distinction between ABMR and TCMR pathophysiology and histology, in many cases, ABMR and TCMR lesions co-occur ("mixed rejection"), and also intermediate forms exist and overlap, often complicating the interpretation of the histological picture of ABMR, even early after transplantation [30,31].

If the DSA are already present prior to transplantation, early ABMR can be seen (sometimes called "type 1" ABMR). However, DSA can also occur *de novo* after transplantation, giving rise to pathological changes of active ABMR at a later stage ("type 2" ABMR) sometimes years post-transplant. Although the underlying pathophysiology of both presentations of ABMR is considered to be similar, *de novo* DSA causing ABMR are more often directed against class II HLA molecules than pre-existing DSA [32], and also the histological presentation of ABMR with pre-existing or *de novo* DSA differs. Biopsies with ABMR due to *de novo* DSA have more transplant glomerulopathy lesions, more tubulitis and concomitant TCMR, more atrophy and fibrosis, and more arteriolar hyalinosis, but similar levels of microcirculation inflammation and C4d deposition, compared to ABMR caused by pre-existing DSA [32,33].

These histological differences between ABMR caused by *de novo* versus pre-existing DSA seem to be primarily related to the important impact of time on the histological presentation of ABMR. A main difference with TCMR, and likely also the reason for its very deleterious impact on outcome, is the inherently chronic time course of ABMR. This is due to the fact that the culprit pathogenic antibodies are notoriously difficult to remove with therapy. The hallmark of this chronicity is transplant glomerulopathy, which typically appears months after the onset of ABMR [34], although also nonspecific arteriosclerosis and IFTA have been associated with ABMR [14,35]. The phenotype of ABMR can therefore differ considerably between patients, reflecting the duration of antibody action and thus time post-transplantation [30]. Early and active ABMR mainly presents with peritubular capillaritis and glomerulitis as primary histological hallmarks, while longer existing and late ABMR presents with transplant glomerulopathy and arteriosclerosis but can still have features of active ABMR (Fig. 2) [30,34].

Antibody-mediated rejection can present subclinically in protocol biopsies [13]. In presensitized patients, with DSA at the time of transplantation, subclinical rejection is detected in almost 50% of protocol biopsies performed at 3 months post-transplantation [13]. In patients with *de novo* DSA, active ABMR is present in 25% of cases at time of detection, and 1 year later in 53%, which illustrates that the injury process caused by DSA does not wane over time [36]. Subclinical ABMR is independently associated with an important increase in the risk of graft loss and is much more deleterious than subclinical TCMR [13].

Despite this high incidence of "type 1" ABMR in patients with pretransplant DSA and its association with outcome, it was recently described that *de novo* occurrence of DSA and herewith associated ABMR leads to even worse graft survival [33]. The reason for the worse outcome with *de novo* DSA compared to pre-existing DSA is unclear [33,37–39] but could be attributed to differences in the antibody characteristics (including the HLA antibody class), with presensitized clones operating before immunosuppression is installed being more amenable to suppression and to fast detection and treatment of early ABMR. It is also plausible that ABMR from *de novo* DSA is often detected later, at a stage when chronic injury already occurred, which leads to lower chances of success of treatment. In addition, *de novo* antibodies, arising under immunosuppression, might be less targetable by additional immunosuppression than antibodies occurring in nonimmunosuppressed individuals [33]. Finally, and importantly, also nonimmunologic factors like nonadherence could be responsible for the occurrence of DSA, but also for the deleterious outcome of patients with *de novo* DSA [30].

Polyomavirus-associated nephropathy

Polyomavirus causes interstitial nephritis, histologically characterized by tubulitis ("t") and interstitial inflammation ("i"), indistinguishable from the tubulo-interstitial inflammation of TCMR. The tubulo-interstitial inflammation of polyomavirus-associated nephropathy (PVAN) is most prevalent between 3 months and 2 years post-transplantation, after which PVAN becomes very rare (Fig. 2) [4,40]. In the meantime, this inflammation induces the development of IFTA [14]. The risk for PVAN early after transplantation likely relates to the increased overall immunosuppression that is provided in the first time post-transplant, which is supported by the finding that the systematic reduction in tacrolimus doses associated with a significant

reduction in the prevalence of PVAN at 1 year [41]. In 1-year post-transplantation protocol biopsies, only 2.5% (26 of 1001 biopsies) shows viral nephropathy [13], which is likely the consequence of improved detection and screening, and timely immunosuppression adaptations. With systematic screening for polyomavirus replication, early detection of PVAN, and rapid adaptation of the immunosuppressive protocol, the impact of PVAN on outcome can be drastically improved [40].

De novo *and recurrent glomerular diseases*

The prevalence of *de novo* or recurrent glomerular disease increases slowly over time after transplantation (Fig. 2), being present in 8% of protocol biopsies at 1 year [41] and reaching a cumulative incidence of 42% by 10 years after transplantation [42]. Glomerular diseases have a histological presentation that is similar to what is seen in native nontransplanted kidneys. Clearly, the occurrence of primary glomerular diseases after transplantation is an independent risk factor for graft failure [43]. It is beyond the scope of this review to discuss the different primary glomerular diseases in more detail. We refer to recent reviews on this topic [42,44].

Chronic lesions

Chronic lesions can be present in all renal compartments (tubulo-interstitial, vascular, and glomerular) and are considered to be irreversible. Chronic lesions in kidney allograft biopsies are complex due to their co-existence and nonspecificity. Most chronic lesions (except transplant glomerulopathy, which is a unique pathologic entity with specific prognostic implications) occur together and are therefore collinear in statistical analyses, making it difficult to analyze them separately [45]. This co-occurrence of chronic lesions in the same biopsies can be caused by a snowball effect, with one lesion exacerbating other lesions and escalating damage. In addition, several chronic lesions share a common pathophysiology, leading to their co-occurrence and nonspecificity. Finally, the inherent association between the cumulative prevalence of chronic lesions and time after transplantation could also be the reason for the co-occurrence of chronic lesions, without a direct pathophysiologic link between them. The co-occurrence of the different chronic lesions is likely a combination of these three phenomena, which makes investigating them separately very difficult, if not impossible. Yet, in this review, we try to elucidate the causes and impact of the different chronic lesions separately.

Interstitial fibrosis and tubular atrophy

Tubular atrophy and fibrosis are very often occurring together, with a correlation coefficient of 0.90 between the atrophy and fibrosis lesions scores [46]. Mild IFTA can already be present at the time of transplantation, especially in older donors [47]. New-onset or progressive tubulo-interstitial damage is very frequent after transplantation [1,3,4,15,28,48–50]. IFTA is multifactorial and can be due to ischemic injury from transplantation, clinical rejection or ongoing (under-recognized) subclinical rejection (see above), calcineurin inhibitor nephrotoxicity, aging, infection (e.g., urinary tract infection, polyomavirus nephropathy, and cytomegalovirus), chronic ischemia (e.g., renal artery stenosis, size discrepancy in pediatric transplantation), chronic postrenal obstruction, and diabetes mellitus. IFTA is the final pathway of nephron injury with its fibrotic healing response, rather than a specific diagnostic entity [3,51,52].

Given that several principal causes of IFTA occur mainly early after transplantation, rapidly increasing scores of IFTA are mostly noticed in the early period after transplantation, although less rapid in recent years than in the earlier cyclosporine era [3,19]. Later after transplantation, progression of IFTA is likely caused by new and ongoing injury from active and progressive diseases. Although it is shown that about 60% of biopsy samples with IFTA after 1-year post-transplantation have an underlying progressive disease such as ABMR or glomerulonephritis [4,6], it remains impossible to relate the chronic tubulo-interstitial damage with only this disease process, while other concurrent diseases like (subclinical) TCMR could also contribute to this chronic injury (see above).

The association of IFTA with outcome, independent of active disease processes and graft function [53], suggests that this chronic injury leads to decreased functional reserve and impaired resistance to novel injuries, progression on the local level even after the resolution of the initial injury process [52]. The occurrence of IFTA should however not lead to the conclusion that fibrosis is in itself the underlying disease process. In the presence of progressive fibrosis, it remains important to elucidate underlying causes and target these. Nevertheless, targeting fibrogenesis directly could also become a valuable strategy in the future to improve graft outcome [52,54].

Arteriolar hyalinosis and CNI nephrotoxicity

It is stipulated that CNI use leads to progressive arteriolar hyalinosis (Banff "ah" score) causing vascular narrowing,

which in return leads to ischemic glomerulosclerosis and tubulo-interstitial damage. Histopathologic lesions of calcineurin inhibitor nephrotoxicity and ah are not specific [5]. Arteriolar hyalinosis is also present in the absence of calcineurin inhibitors and can be present in baseline biopsies, from donor origin [47,55,56]. Early arteriolar hyalinosis is often mild and patchy compared with later arteriolar hyalinosis, which is persistent and progressive. By 10 years after transplantation, ah is virtually universal and present in more up to 90% of protocol biopsies [3,57,58]. This is similar in cyclosporine-treated and tacrolimus-based patients [19] and was a big area of concern an interest in the first years of the 21st century, where calcineurin inhibitor minimization and elimination were extensively studied in numerous clinical trials, with mixed results (better renal function but higher risk of rejection, without benefits on graft survival) [59].

Despite this high prevalence of ah over time after transplantation and the association with toxicity of the calcineurin inhibitors, the exact effect of ah on outcome is unclear. An association between ah and decreased graft failure has been described in for-cause biopsies [4,60,61]. A possible explanation for this unexpected finding could be the adequate exposure to CNIs, leading to arteriolar hyalinosis but protecting against rejection, which is strongly supported by a more recent analysis [62]. On the other hand, older donor age associates with arteriolar hyalinosis in baseline biopsies but also with *de novo* onset or progression of ah after transplantation, thus possibly increasing the susceptibility of older donor kidneys to chronic injury [5].

The interpretation of ah should therefore take into account the timing of its occurrence: early after transplantation ah likely reflects donor pathology, while later the absence of hyalinosis should lead to the suspicion of (potentially deleterious) under-immunosuppression in patients treated with calcineurin inhibitors.

Glomerulosclerosis

Another lesion with known time-dependent occurrence is glomerulosclerosis. In transplant centers with a large proportion of older donors, significant numbers of baseline biopsies already have >10% of glomerulosclerosis [47,56]. The percentage glomerulosclerosis post-transplantation should therefore be interpreted in light of the donor characteristics and ideally the histology of baseline biopsies.

Glomerulosclerosis after transplantation is considered a fundamentally secondary lesion, highly nonspecific and thus not relevant for differential diagnostics of renal allograft pathologies. In an earlier study, Nankivell *et al.* [43] described a tri-phasic nonlinear time course of glomerulosclerosis after transplantation, starting with an intense but limited peak in the first month, and related this to cold ischemia and early structural CNI nephrotoxicity. As this was a short-lived peak in the occurrence of glomerulosclerosis, damage and impact on the long term remain limited. This early phase was followed by a quiescent phase up to 2 years despite ongoing tubulo-interstitial and immunologic damage in this time period. The tubulo-interstitial damage occurring in this period (see above), however, is proportional to the extent of later glomerulosclerosis, which suggests that a-tubular glomeruli are prone to become globally sclerosed. In addition, progressive glomerulosclerosis increased with subclinical rejection, independent of tubular damage. A third phase of glomerulosclerosis was related to arteriolar hyalinosis (ah), suggested to be secondary to CNI nephrotoxicity (see above). The higher the grade of arteriolar hyalinosis, the more ischemic glomerular loss was noted, which was explained by cyclosporine effects on vascular narrowing, subsequent arteriolar hyalinosis, and ensuing glomerular hypoperfusion [43].

In a more recent study by the same group, however, the time course of glomerulosclerosis seems to be different to what was previously described, with a more constant rate of development. Here, a greater increase in glomerulosclerosis scores with the presence of higher grades of arteriolar hyalinosis lesions was noted. A greater severity of hyalinosis associated with increased glomerulosclerosis in sequential biopsy pairs irrespective of the CNI used, which suggests a hemodynamic cause (ischemic glomerulosclerosis) [19,63]. Also other glomerular disease processes, such as transplant glomerulopathy and *de novo* or recurrent glomerular diseases, could ultimately lead to global glomerulosclerosis, as is also the case in native kidney diseases [42,64]. Finally, further work is necessary to elucidate the potential involvement of other phenomena in progressive glomerulosclerosis, such as the concept of glomerulosclerosis due to tubular atrophy (a-tubular glomeruli).

Very few studies take the percentage glomerulosclerosis into account in outcome prediction, likely because glomerulosclerosis is not included in the Banff classification and therefore remains under reported. We recently demonstrated a highly significant association between glomerulosclerosis, proteinuria, and graft outcome in univariate analysis, but glomerulosclerosis was not retained in multivariate models [43,47,65], which

supports the concept that glomerulosclerosis is a secondary phenomenon and not an independent factor in graft outcome. More systematic inclusion of glomerulosclerosis in studies reporting on renal allograft histology, and in predictive models, seems necessary to elucidate the relevance of this lesion for prognostication (see below).

Transplant glomerulopathy

Transplant glomerulopathy results from endothelial remodeling after sustained antibody-mediated injury, which leads to multilayering and double contours of glomerular basement membrane [65]. Transplant glomerulopathy (Banff "cg" score) is included in the criteria for chronic antibody-mediated rejection [33]. Cg increases over time, but its prevalence is very divergent between groups, largely because of the variable immunologic risk profile. Although transplant glomerulopathy is often used as hallmark of chronic ABMR, also this lesion is nonspecific, and glomerular basement membrane double contours can also reflect other processes like recurrent membranoproliferative glomerulonephritis.

The prevalence of cg lesions in renal allograft biopsies differs between different centers, and ranges from 6.7% to 20% in the first years [1,57,66–68], while larger studies with longer duration showed that cg was reached a much higher prevalence up to almost 70% by 10 years after transplantation in protocol and indication biopsies [4,43]. Median time from transplant to biopsy findings of cg ranges between 5.5 and 7.1 years [65,67,68]. The later in time cg is identified, the more severe the grade and irreversibility, which is reflected in worse outcome [65]. As discussed above, this is one of the likely explanations why ABMR due to *de novo* DSA has worse outcome than early ABMR due to pre-existing DSA. With more chronicity and more cg, the process of ABMR becomes irreversible and nonresponsive to treatment. This fits in the concept that chronic ABMR is the result of a progressive disease with cumulative injury over time due to continuing antibody activity, rather than a one-hit phenomenon. The finding that prior rejection episodes were present in 70% of cases with transplant glomerulopathy, with most being ABMR (53%), and fewer being mixed (10%) or TCMR (5%) [68], as well as several animal models [69–71], corroborates this concept.

Arteriosclerosis

Arteriosclerosis is an elementary lesion of kidney allografts that is less well studied. In baseline biopsies, arteriosclerosis or vascular intimal thickening (Banff "cv" score) is only present in a very small percentage (4.3%) [47]. After transplantation, arteriosclerosis prevalence increases rapidly. In 1-year protocol biopsies, up to 34% of protocol biopsies show moderate to severe chronic vascular damage (cv2-3) [35]. Vascular intimal thickening (cv) in implantation biopsies is related to donor age and cv lesions develop faster in kidneys of older donors [5].

Next to traditional cardiovascular risk factors and donor pathology, circulating anti-HLA antibodies are major independent determinants of severe arteriosclerosis [35,69,72]. The importance of DSA and ABMR for arteriosclerosis is likely explained by the fact that the vascular endothelium, obviously present in arteries, is the primary target of circulating allo-specific antibodies. The onset of ABMR-associated arteriosclerosis (or "transplant vasculopathy") is not necessarily preceded by vasculitis or intimal arteritis ("v"). Although transplant vasculopathy is often suggested to be different from "banal" arteriosclerosis, the distinction between antibody-induced changes and changes due to, for example, hypertension cannot be made easily, certainly after disappearance of the rejection-associated vascular hypercellularity over time or with treatment. Rapid onset of transplant vasculopathy should therefore instigate a detailed study of the immune profile of the transplantation and a search for the presence of DSA or uncontrolled cardiovascular risk factors.

The impact of graft arteriosclerosis on outcome after transplantation is not clear. We recently showed that arteriosclerosis in indication biopsies associates with graft failure, but not when arteriosclerosis is detected in protocol biopsies [4,60]. This is likely explained by the finding that only DSA-induced arteriosclerosis associates with graft outcome, but not arteriosclerosis in the absence of DSA [42].

Mesangial matrix expansion

Mesangial matrix expansion (mm) increases rapidly until 1 year after transplantation, with a slower but still progressive course thereafter [43]. Mm correlates with tubular atrophy, interstitial fibrosis, transplant glomerulopathy, and arteriolar hyalinosis. Mesangial matrix expansion adjusted for time after transplantation is less in patients treated with mycophenolate [43]. Its causes and impact on graft outcome are not well established and need further study.

Associations do not prove causality: complex biopsies

As is evident from the above description of the time-dependent evolution of lesions and histological diagnoses, and their mutual pathophysiologic interactions, renal allograft biopsy histology is often complex, which causes difficulties in interpretation. Active disease processes co-occur with extensive chronic damage, especially in biopsies performed late after transplantation and in noncompliant patients (Fig. 1).

The question whether chronic damage relates to the concomitant or previous specific disease processes remains difficult to answer in individual cases, due to progression or waning of disease processes over time. Moreover, subclinical processes of rejection and inflammation are important in the establishment of chronic damage but are by definition missed when no protocol biopsies are performed, or when the subclinical injury processes are active away from the protocol biopsy timing. We therefore have to be careful not to oversimplify the interpretation of late biopsy histology and remain open to the idea that specific diseases can precede chronic lesions that are not caused by these earlier specific diseases, but that are the consequences of even unnoticed other disease processes. We also need to take into account that chronic lesions that are diagnosed together with specific diseases are not necessarily caused by these concomitant specific diseases. Associations do not prove causality.

Outcome and prognosis

Validated prognostic markers do not exist in the field of transplantation. A true prognostic marker should have clinically relevant sensitivity and specificity, positive predictive, and/or negative predictive value. Prognostic markers that represent bad outcome could be used to provide patients and their doctors a glimpse of the future, to guide the frequency of clinical follow-up, guide therapeutic decisions, to establish inclusion and exclusion criteria for interventional studies, and if validated sufficiently, to function as surrogate end points in clinical studies. As we outlined above, the association of histological lesions and diseases with outcome depends on time post-transplantation, where the progressive chronic injury that ensues from the active disease processes is sometimes more important than the disease activity itself.

The important correlations between the different histological lesions and diagnoses, and the dependency on time after transplantation, suggest that individual histological lesions or diagnoses will not be sufficiently good prognostic markers. It is likely that only in combination with clinical parameters such as glomerular filtration rate or proteinuria, histology can be used for prognostication. In addition, it needs to be evaluated whether time after transplantation is important for establishing an individual patients' prognosis after diagnosis of allograft injury. Adding a multidimensional prognostic layer to the current diagnostic Banff classification, including graft functional characteristics and timing, could become an interesting aid in the interpretation of complex histological lesions and mixed diagnoses, and in therapeutic decision-making.

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Conflicts of interest

The authors have declared no conflicts of interest.

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