Glomerular macrophage index (GMI) in kidney transplant biopsies is associated with graft outcome

Johan Mölne1,2 | Salmir Nasic3,4 | Verena Bröcker1,2 | Bernd Stegmayr5 | Marie Felldin6 | Björn Peters3,7

1 Institute of Biomedicine, Department of Laboratory Medicine, University of Gothenburg, Gothenburg, Sweden
2 Clinical Pathology, Sahlgrenska University Hospital, Gothenburg, Sweden
3 Department of Molecular and Clinical Medicine, Institute of Medicine, the Sahlgrenska Academy at University of Gothenburg, Gothenburg, Sweden
4 Research and Development Centre, Skaraborg Hospital, Skövde, Sweden
5 Public Health and Clinical Medicine, Umeå University, Umeå, Sweden
6 Department of Transplantation University of Gothenburg, Gothenburg, Sweden
7 Department of Nephrology, Skaraborg Hospital, Skövde, Sweden

Correspondence
Björn Peters, University of Gothenburg, the Sahlgrenska Academy, Institute of Medicine, Department of Molecular and Clinical Medicine, 41 345 Gothenburg, Sweden. Email: bjorn.peters@gu.se

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Abstract
Background: Macrophages in renal transplants have been shown to participate in antibody-mediated rejection and are associated with impaired renal function. We calculated the glomerular macrophage index (GMI) in a large transplant biopsy cohort, studied its quantity in different diagnostic groups, to clarify its possible impact on graft survival.

Methods: GMI, defined as the mean number of macrophages in 10 glomeruli, was prospectively quantified in 1440 renal transplant biopsies over a 10-year period. The main histopathological diagnoses were grouped into eight disease entities, and GMI was compared to normal transplant biopsies as the reference group. The impact of GMI on graft survival was analyzed.

Results: GMI was highest in chronic (mean 9.4) and active (9.7) antibody mediated rejections (ABMR), mixed rejections (7.6), and recurrent or de novo glomerulonephritis (7.5) and differed significantly from normal transplants (1.3) in almost all diagnostic groups. Hazard ratios for graft loss were significantly increased for all biopsies with GMI ≥ 1.9 compared to GMI < 0.5 (reference group) in an adjusted Cox regression model and increased with higher GMI levels. Biopsies with GMI ≥ 4.6 had < 60% 10-year graft-survival, compared to > 80% with GMI ≤ 1.8.

Conclusion: GMI levels were predictive of graft loss independent of histological diagnoses and may guide clinicians to decide follow-up and therapy.

KEYWORDS
glomerular macrophage index, graft outcome, macrophage, rejection, renal transplant biopsies

1 | INTRODUCTION

Today kidney transplantation is a routine procedure that enables many patients with renal failure to live nearly normal lives. Acute T cell-mediated rejection (TCMR) is less common today, partly due to optimized induction therapy, and can usually be treated successfully with little or no impact on graft outcomes.1 In contrast, chronic rejections and especially chronic antibody-mediated rejection (ABMR), often as a result of iatrogenic or patient-led insufficient immunosuppression,2 are difficult to control. Furthermore, recurrent glomerular diseases,3 side effects of drugs,4 human leukocyte antigens (HLA), other antigen mismatches,5,6 and many other factors lead to organ dysfunction in
the long term. Therefore, it is important to identify prognostic markers to detect renal transplant insults, especially immunological, to prevent overtreatment and insufficient immunosuppression to improve the outcome of renal transplantation.

Macrophages are important cells that participate in both the innate and adaptive immune reactions. In the present study, we included both monocytes and macrophages under the term macrophages. In the transplant setting, macrophages are involved both in the immediate innate response due to ischemia-reperfusion injuries and in early and late transplant alloresponses, since there are foreign antigens, mainly HLA-antigens, driving the recipient immune response against the transplant. Macrophages and dendritic cells engulf donor antigens and present them to the immune system, mainly to CD4+ T helper cells, thus initiating an immune response. Macrophages have been investigated and monitored for a long time in renal transplant biopsies. The number of macrophages increases in renal cortical tissues during ischemia as well as in acute and chronic TCMR and ABMR. Glomerulitis is a well-established criterion for ABMR, and a high number of glomerular macrophages has been shown to correlate with renal transplant rejection and worse prognosis, mainly in ABMR. The glomerular macrophage index (GMI), the average number of glomerular macrophages in a biopsy, was first investigated by Magil et al. in native glomerular diseases and later in transplants.

The aim of our study was to investigate the level of GMI in a large cohort of post-transplantation biopsies and to determine the impact of GMI on kidney transplant outcomes. To this end, GMI was consecutively and prospectively calculated in all transplant biopsies obtained over a 10-year period at our transplant center.

## 2 MATERIAL AND METHODS

### 2.1 Renal biopsies and study design

Our Regional Renal Biopsy Registry included 3130 transplant biopsies from 1542 unique patients obtained between January 1, 2007, and follow-up until September 30, 2017. As the outcome in the analyses was graft survival, we decided to include only the first biopsy (first registered biopsy). The majority of biopsies were on clinical indication since protocol biopsies were only performed in clinical studies at our center and represented only 6% of the material. Of the 1542 patients 36% were women and the median age at biopsy for all patients was 52 years (interquartile range [IQR] 40–61 years). Thirty-six percent of the patients received a kidney from a deceased donor and 37% from a living donor. The patients had received their first kidney transplant between 1977 and 2017, of which 90% were transplanted after 1998 and 50% after 2008. The median time between transplantation (Tx) and the first registered transplant kidney biopsy was 4.6 months (IQR 0.5–74 months). After excluding 80 patients due to incomplete data, there were 1442 patients. GMI was missing in another 22 patients due to lack of cortex or globally sclerosed glomeruli only, and the data in Table 1 is based on 1440 biopsies.

Data were collected in a quality assessment registry (TIGER) that includes all kidney-transplanted patients at Sahlgrenska University Hospital in Gothenburg, Sweden from 1965 onwards. A limitation of this registry is that data have to be registered manually at each separate hospital, and due to shortage of local resources some data are missing. The TIGER data were merged with data from the Regional Renal Biopsy Registry, which collected data from January 1, 2007. The biopsy registry and TIGER data analyzed in the study included follow-up data and outcomes until September 2017. Informed Consent was obtained and the Regional Ethical Review Board (DNR: T586-14, EXP 2014-07-24) in Gothenburg, Sweden approved the study.

### 2.2 Histopathology and immunohistochemistry

Biopsies were fixed in 4% buffered paraformaldehyde ("formalin"), transported to the laboratory, post-fixed under constant shaking for at least 1 h, embedded in paraffin, sectioned (3–4 μm), and stained with hematoxylin-eosin, periodic acid Schiff, Ag-Jones, trichrome, and elastin van Gieson. All biopsies were immunostained as previously described for CD68 (Dako, N1576, Copenhagen, Denmark) and C4d (Abcam, ab 58781, Cambridge, UK) using an automated procedure, the EnVision™ Flex High pH (Link) detection kit (Dako K8000). C4d was evaluated in cortical peritubular capillaries and in medullary vasa recta, when cortex was missing. In addition, all biopsies were routinely stained for CD3, CD20, and SV-40 (polyomavirus) for the first post-transplant year and thereafter also for IgM and C5b-9. When indicated by light microscopic findings or clinical information, such as proteinuria, additional staining for IgG, IgA, C1q, C3, and light chains were done. Biopsies were scored using the Banff classification from 2007 and updated accordingly. The diagnosis chronic allograft nephropathy was used until 2009 and was thereafter omitted in favour of IFTA (interstitial fibrosis and tubular atrophy of uncertain cause). To enable group comparisons, the final histopathological diagnoses were divided into eight disease groups and one group of transplant biopsies with no major significant pathologic findings that was used as a reference group (Table 1). Biopsies categorized as minor abnormalities had mild IFTA and no other active disease. Chronic changes included moderate and severe IFTA, nephroclerosis, chronic calcineurin inhibitor (CNI) toxicity and hydropnephrosis. Hematological diseases included posttransplant lymphoproliferative disease and amyloidosis. All glomerular diseases with an immunological pathogenesis (IgA nephropathy, membranous glomerulonephritis, post infectious GN, membranoproliferative GN, focal segmental glomerulosclerosis, FSGS, vasculitis and lupus nephritis) were grouped as GN while mainly diabetic glomerulopathy and 12 cases with glomerulopathy of unknown cause were grouped as glomerular diseases without GN. The rejection group was further subdivided into active or chronic ABMR, acute or chronic TCMR, and mixed cases (Table 1), based on the effective Banff-classification at the time of biopsy. Subspecialized renal pathologists reported all biopsies, and the final diagnoses were retrieved from the local pathology database without re-examination.
## TABLE 1
Levels of GMI in relation to various patient characteristics at first kidney transplant biopsy (total n = 1440)

| Variables                        | Mean (SD) | Median (Q1-Q3) | p-valuea |
|----------------------------------|-----------|----------------|----------|
| **Gender**                       |           |                |          |
| Male, n = 913                    | 3.6 (5.1) | 1.7 (.9-3.7)   | .051     |
| Female, n = 527                  | 4.6 (6.2) | 2.0 (8.5-9.9)  |          |
| **Age group**                    |           |                |          |
| ≤40 years, n = 370               | 3.2 (4.2) | 1.7 (.8-3.6)   | .065     |
| 41–50 years, n = 302             | 4.1 (5.6) | 2.0 (9-5.0)    |          |
| 51–60 years, n = 360             | 4.0 (5.6) | 1.8 (9-4.5)    |          |
| ≥60 years, n = 408               | 4.4 (6.3) | 1.9 (9-5.0)    |          |
| **C4d degree**                   |           |                |          |
| C4d 0 (negative), n = 1248       | 3.5 (5.1) | 1.6 (.8-3.6)   | <.001*   |
| C4d 1–3, n = 192                 | 7.0 (7.1) | 5.1 (1.8-10)   |          |
| **Time from Tx to biopsy**       |           |                |          |
| <6 months, n = 700               | 3.2 (5.2) | 1.4 (.8-3.0)   | reference|
| 6–24 months, n = 163             | 2.6 (4.0) | 1.3 (7-2.4)    | .200     |
| 2–6 years, n = 145               | 5.4 (7.2) | 2.4 (1.1-6.8)  | <.001*   |
| >6 years, n = 336                | 5.3 (5.9) | 3.0 (1.4-6.8)  | <.001*   |
| **Unknown date of transplantation (n = 96)** | | | |
| **Diagnostic groups**            |           |                |          |
| Normal biopsy findings, n = 85    | 1.3 (1.0) | 1.0 (5.1-9)    | reference|
| Infections and tubulointerstitial nephritis (TIN), n = 61 | 2.1 (3.4) | 1.0 (5.2-1)    | .924     |
| Acute tubular injuries, n = 177   | 2.6 (3.6) | 1.3 (8-2.7)    | .018*    |
| Chronic changes including IFTA, n = 330 | 2.6 (3.2) | 1.5 (8-2.9) | .001*   |
| Hematological diseases, n = 5     | 2.8 (1.3) | 2.4 (2.0-2.8)  | .009*    |
| Glomerular diseases, n = 82       | 6.3 (6.5) | 4.0 (1.7-8)    | <.001*   |
| GN, recurrent/de novo, n = 48     | 7.5 (6.8) | 5.2 (2.5-12.0) | <.001*   |
| Glom disease, no GN, n = 34       | 4.6 (5.8) | 2.4 (1.5-5.5)  | <.001*   |
| Minor abnormalities, n = 149      | 1.9 (3.5) | 1.0 (6.1-9)    | .902     |
| Borderline changes, n = 126       | 3.6 (4.7) | 2.0 (9-4.4)    | <.001*   |
| Rejections, n = 425               | 6.7 (7.5) | 3.9 (1.5-9.2)  | <.001*   |
| Acute TCMR, n = 234               | 5.2 (6.9) | 2.3 (1.2-6.5)  | <.001*   |
| Chronic TCMR, n = 12              | 6.3 (9.9) | 3.1 (1-7.2)    | .026*    |
| Active ABMR, n = 28               | 9.7 (9.1) | 6.5 (3.7-13.2) | <.001*   |
| Chronic ABMR, n = 69              | 9.4 (7.6) | 7.0 (3.7-13.4) | <.001*   |
| Combined active ABMR and acute TCMR, n = 5 | 6.7 (7.7) | 1.6 (1.2-15.0) | .186     |
| Combined chronic ABMR and chronic TCMR, n = 77 | 7.6 (6.9) | 6.5 (2.0-10.5) | <.001*   |

Note: Q1 = first quartile, Q3 = third quartile.

*aComparisons by Mann-Whitney for two groups and Kruskal-Wallis test for more than two groups.

*bPairwise comparisons by Mann-Whitney test with normal biopsy findings as the reference group.

*cAcute tubular injuries = acute tubular necrosis (ATN) and acute CNI-toxicity (calcineurin inhibitor).

*dChronic changes including chronic CNI-toxicity and IFTA/CAN; IFTA = interstitial fibrosis and tubular atrophy.

*eIncluding transplant glomerulopathy, n = 30; CNI, calcineurin inhibitor; GN, glomerulonephritis.

*Statistically significant difference.
2.3 GMI-calculation

All renal transplant biopsies sent to our laboratory for histopathology were scored for GMI at the time of routine biopsy reporting. Biopsies containing insufficient material (n = 22: medullary tissue only, no or sclerotic glomeruli only) were excluded resulting in 1440 biopsies remaining for analysis. When present, ten glomeruli were evaluated systematically, always starting at the outermost end of the cores, following the biopsy cores until 10 glomeruli were scored. Ten glomeruli were chosen as it is a number possible to count in clinical practice. Further, a sub analysis of random biopsies (n = 94) showed no additional value in counting all glomeruli in the biopsy (mean 2.7 for 10 glomeruli compared to 2.8 for all glomeruli, data not shown). Globally sclerotic glomeruli were excluded. CD68-positivity, using high-power field (HPF, 400 ×), was counted as a cell when containing a nucleus in the section plane or showing a rounded structure in keeping with a cell body. Cellular processes or immunopositive small dots were not counted (Figure 1). To obtain the GMI value, the total number of positive cells was divided by the number of glomeruli obtained. Furthermore, at least 10 glomeruli were identified in 78% of all cases, four to nine glomeruli in 16%, and two to three in 6%. Sensitivity analysis (data not shown) revealed no differences in graft survival when biopsies with <10 glomeruli were excluded; therefore, all GMI values were included in the final analysis.

2.4 Statistics

Based on GMI, the data were split into eight evenly distributed classes. The number of classes was determined based on the intention to construct a number of classes that vary in the level of GMI and, at the same time, obtain classes large enough with respect to the number of patients to optimize statistical power. These classes, representing different levels of GMI, were later used to explore possible associations between GMI and graft survival.

3 RESULTS

3.1 GMI and diagnosis at the first transplant biopsy

GMI was significantly different between the diagnosis groups, and in pairwise comparison for most diagnoses, GMI differed significantly from normal biopsies (Table 1). GMI was highest in chronic ABMR (median GMI = 7.0), active ABMR (median = 6.5) and
glomerulonephritis, recurrent and de novo (median = 5.2); these were followed by TCMR (chronic TCMR median = 3.1, acute TCMR median = 2.3) and other glomerular diseases (median = 2.4). P-values were < .001 for all comparisons except for chronic TCMR (p = .026), with normal biopsies as the reference (Table 1). In contrast, infections and tubulointerstitial nephritis (including polyoma virus nephropathy), chronic damages, and hematological diseases (five cases only) showed low GMI (Table 1). The GMI did not differ with regard to gender or age (Table 1). The levels of GMI in the diagnostic groups are illustrated in Figure 2. To test if the maximum number of macrophages per glomerulus in a biopsy (G-max) is more informative than GMI, a sub-analysis was performed on a subset of biopsies (n = 320; normal biopsy findings, glomerular diseases, borderline changes and rejections). Graft survival and distribution between the diagnostic groups showed the same results as for GMI (data not shown). Living donors were compared with deceased donors with respect to GMI in a univariate test and there was no statistically significant difference (p-value = .264).

3.2 | C4d positivity and GMI

Of the 1440 transplant biopsies, 1248 (87%) were negative for C4d. Positivity (grade 1–3) was found in 192 (13%) of the transplant biopsies, and GMI was significantly higher in these biopsies with a median of 5.1 compared to 1.6 for C4d negative biopsies, p < .001 (Table 1).

3.3 | Graft survival according to GMI-levels

A Kaplan-Meier analysis was performed for graft-survival (Figure 3) and GMI-levels as possible explanatory variable. The analysis revealed a statistically significant association between GMI levels and graft survival. Low GMI values ≤ .5 had the best 10-year graft-survival (≈ 90%), GMI-levels .6 to 1.8 had the second best survival (80%–90%), GMI 1.9–4.5 had an intermediate survival (70%–75%), and GMI ≥ 4.6 had the lowest 10-year graft-survival (50%–60%) (Figure 3). Further, a sub-analysis showed that GMI ≥ 4.6 or higher had a worse graft survival already at 1 year of follow-up (data not shown).

In the next step, a Cox regression was performed and graft survival was analyzed in relation to GMI levels, and the HR is presented (Table 2). The risk of graft loss increases with increasing GMI. HR was significantly higher for patients with a GMI of 1.9–2.7 (HR 2.9, p = .002), GMI 2.8–4.5 (HR 3.3, p < .001), GMI 4.6–9.3 (HR 5.4, p < .001), and GMI ≥ 9.4 (HR 6.3, p < .001) compared to GMI < .5 as the reference group. In the Cox model, we controlled for age at biopsy, gender, C4d degree, histopathological diagnosis, and the time from transplantation to biopsy (Table 2). An additional observation was that GMI was slightly higher in early transplant biopsies (<1 month), lowest between 1–6 months and thereafter increased with time (Figure 4). As time from transplantation to biopsy seemed to influence the association between GMI and graft survival and more precisely as magnitude of the impact of GMI on graft survival seemed to differ depending on time between transplantation and biopsy we further explored interaction between time from transplantation to biopsy and GMI in a cox- model with graft survival as outcome. Stratifying on different time periods we saw that pattern of association between GMI and graft survival was different for biopsies 6 months after transplantation compared to biopsies within 6 months after transplantation. Therefore, we used 6 months as cut-off point when analyzing the interaction and the interaction term "GMI-category*time between transplantation and biopsy" was statistically significant with p-value = .005. We saw that impact of increasing GMI was more obvious among patients biopsied...
FIGURE 3 Kaplan-Meier estimates of death-censored graft survival after biopsy, according to GMI-levels at biopsy. Increasing levels of GMI showed a statistically significant association with worse graft outcomes. Low levels (<1.9) have a 10-year graft survival of >80%, intermediate levels (1.9–4.5) have a graft survival around 70%, and GMI ≥ 4.6 have a 10-year graft survival between 50% and 60%. Further, a GMI ≥ 4.6 predict poor graft survival already 1 year after biopsy.

TABLE 2 Risk for different end-points according to GMI-class at biopsy

| GMI classes | Crude association | Adjusted association | p-value | p-value |
|-------------|-------------------|---------------------|---------|---------|
|             | HR with 95% CI    | p-value             | HR with 95% CI | p-value |
| ≤.5 (n = 205) | reference         | –                   | reference | –       |
| .6-.9 (n = 191) | 2.09* (1.03-4.24) | .042                | 1.98 (.97-4.04) | .059   |
| 1.0-1.2 (n = 157) | 1.47 (.67-3.21)   | .339                | 1.44 (.66-3.17) | .361   |
| 1.3-1.8 (n = 177) | 2.11* (1.04-4.31) | .038                | 1.71 (.83-3.51) | .144   |
| 1.9-2.7 (n = 190) | 3.55* (1.84-6.86) | <.001               | 2.92* (1.50-5.67) | .002   |
| 2.8-4.5 (n = 162) | 4.18* (2.17-8.03) | <.001               | 3.32* (1.70-6.46) | <.001  |
| 4.6-9.3 (n = 183) | 6.63* (3.56-12.38) | <.001               | 5.39* (2.81-10.30) | <.001  |
| 9.4-47.0 (n = 175) | 8.69* (4.69-16.09) | <.001               | 6.33* (3.33-12.04) | <.001  |

Cox regression - Hazard ratio (HR) based on crude (univariate) and model adjusted for covariates where time from biopsy to endpoint was the outcome. Abbreviations: n, number; ref, reference category for calculation of HR; Tx, transplantation.

*Adjusted for age, gender, C4 degree, diagnosis at biopsy, and time from Tx (1344 patients with complete data on all included variables were analyzed in the multivariate Cox regression).

*Statistically significant increase in risk.
FIGURE 4  GMI-levels at different biopsy times after transplantation. The figure shows that GMI increased with time after transplantation but was high in the first month after transplantation. There were statistically significant differences between the GMI levels at different time points after transplantation (p < .01).

4 | DISCUSSION

In this study, we report a significant impact of the number of monocytes/macrophages in renal glomeruli, calculated as a GMI, on transplant outcomes in a large cohort of renal transplant biopsies. GMI was highest in biopsies with active or chronic ABMR, intermediate in those with TCMR, and lower in those with borderline changes. A high index was also found for recurrent glomerulonephritis and other glomerular diseases (Table 1). Further, a GMI ≥ 4.6, and in particularly over 9.4, indicates a significantly worse graft survival (Figure 3). In addition, positive C4d staining in peritubular capillaries was significantly associated with a high GMI, indicating that GMI is associated with ABMR. Our findings confirm those of previous studies conducted in a limited number of patients.8–13,16,19,21

Several early studies of renal biopsies have shown that a high number of macrophages (and T-cells) in renal transplant biopsies is associated with rejection and a worse prognosis.11,12 Early studies focusing on macrophages in glomeruli were performed using histochemical techniques and showed high impact of monocyte infiltration (≥ 2 cells per biopsy or occurring in at least 50% of glomeruli) on graft failure.26,27 Magil established a GMI for glomerular diseases in native kidney biopsies, and the same index was later used in transplant biopsies.13,23,28 Studies in transplant biopsies have used CD68 as a marker of monocytes/macrophages, and this marker is still commonly used. Ozdemir et al. were the first to demonstrate that GMI was high in biopsies with acute and chronic rejection but was low in normal biopsies and biopsies with CNI-toxicity.23 In a series of biopsies with rejection, Magil and Tinckam demonstrated that GMI (and neutrophils) was higher in C4d positive cases.13 They also showed that transplant glomerulitis and C4d positivity in peritubular capillaries correlated with ABMR, whereas C4d negative biopsies correlated with TCMR.14 Papadimitriou et al.21 reported that the maximal number of macrophages in glomeruli, G-max, was associated with ABMR in biopsies obtained > 1 year after transplantation. They could further show that G-max for > 12 CD68 positive macrophages (but not for CD3 positive T-cell glomerular counts) was associated with an increased risk for graft dysfunction.29 We used G-max for analysis on a subset of biopsies (n = 320) from cases with normal biopsy findings, glomerular diseases, borderline changes and rejections, which did not add any information on graft survival compared to GMI (mean). All the mentioned studies, except for those by Papadimitriou et al. (240 and 1101 biopsies), were performed on a fairly small number of patients and have not been followed up by any large studies. Therefore, we calculated GMI in all transplant biopsies from January 1, 2007, to determine whether GMI has any prognostic value. Indeed, we show that a high GMI in the first transplant biopsy is significantly associated with worse prognosis and that GMI varies in different diagnostic groups (Table 1 and Figure 3).

It is difficult to establish a clinically significant level of GMI. Early histochemical studies demonstrated significant differences between few monocytes (two per glomeruli) compared to no monocytes.26,27 Using CD68, some studies have used a level of < 1 compared with > 1 in GMI and showed a difference in graft function14 and graft loss.28 Later studies have shown that GMI levels > 3 were only seen in ABMR and that a GMI > 1.89 correlated with microvascular inflammation, a cardinal sign of ABMR.30 In a sub-analysis of their larger study, Lefaucheur et al.
showed that patients with ABMR and worse outcome had an average GMI of 6.8 (n = 8) compared to GMI 3.2 in an ABMR group (n = 13) with better outcome.

The present large study demonstrates that indication biopsies with normal biopsy findings have a mean GMI of 1.3, while patients with GMI ≥ 1.9 have impaired graft survival. Furthermore, GMI levels ≥4.6 have a worse prognosis, and levels > 9.3 have a substantially worse outcome, measured as graft survival (Figure 3). However, in early transplant biopsies taken within 6 months, the impact of graft survival is only significant for a level of > 9.3. The clinical implication of this finding is that awareness should be given to transplants with high GMI levels. However, the level of GMI that should render increased immunosuppression is impossible to determine at this point. This question can only be answered in a prospective randomized controlled trial with treatment and control arms.

The limitations of the present study include the long study period with variations in immunosuppression protocols, no re-evaluation of biopsies, and the use of registry data. The time after transplantation may be a concern since we included biopsies ranging from within 2 weeks (∼25%) to many years. As shown in Figure 4, GMI values increased with time after transplantation; however, we still found an increased risk of graft loss when corrected for time. The strength of our study is the large number of consecutive and prospective GMI values collected over a 10-year period (Table 1).

It seems well motivated to use histological outcome criteria, including GMI, to help guide further treatment strategies. Our finding that a high GMI level correlates with a shorter expected graft survival suggests that GMI can be a supportive parameter when deciding therapy. Further studies need to address the utility of this parameter and correlate GMI to other established Banff lesion scores, especially glomerulitis.

In conclusion, we show that the GMI in transplant renal biopsies were highest in antibody-mediated and mixed rejections, as well as in de novo and recurrent glomerulonephritis. Further, GMI levels were predictive of graft loss independent of time after transplantation and histological diagnoses and may guide clinicians to decide on intensity of follow up and therapy.

AUTHOR CONTRIBUTION
Johan Mölne instigated the investigation and participated in the research design, research performance, data analysis, and writing of the paper. Salmir Nasic participated in performance of the research, data analysis and manuscript writing. Verena Bröcker participated in the research, data analysis, and manuscript writing. Bernd Stegmayr participated in the research design, research performance, data analysis and manuscript writing. Marie Felldin participated in data analysis and manuscript writing. Björn Peters participated in the research design, research performance, data analysis, and manuscript writing.

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CONFlict OF INTEREST
The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT
The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

ORCID
Marie Felldin https://orcid.org/0000-0003-1618-0272
Björn Peters https://orcid.org/0000-0003-1199-8948

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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