

## A novel immune function biomarker identifies patients at risk of clinical events early following liver transplantation

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**FOOTNOTES PAGE:****Abbreviations:**

AUROC – Area under the receiver operating curve

CNI – calcineurin inhibitor

D – Day

ELISA – Enzyme-linked immunosorbent assay

HCC – hepatocellular carcinoma

HCV – hepatitis C virus

LR – Likelihood ratio

M – Month

QFM – QuantiFERON Monitor

tBPAR – treated and biopsy proven acute rejection

W – Week

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**Abstract:**

Balancing immunosuppression after liver transplant is difficult, with clinical events common. We investigate whether a novel immune biomarker based on a laboratory platform with widespread availability that measures interferon gamma (IFN $\gamma$ ) after stimulation with a lyophilized ball containing an adaptive and innate immune stimulant can predict events following transplantation. 75 adult transplant recipients were prospectively monitored in a blinded, observational study. 55/75 (73.3%) patients experienced a total 89 clinical events. Most events occurred within the first month. Low week 1 (W1) results were significantly associated with risk of early infection (AUROC 0.74,  $p=0.008$ ). IFN $\gamma \leq 1.30$  IU/mL (LR+ 1.93, sensitivity 71.4%, specificity 63.0%) was associated with the highest risk for infection with minimal rejection risk. Nearly half the cohort (27/60, 45.0%) expressed IFN $\gamma \leq 1.30$  IU/mL. Moreover, an elevated W1 result was significantly associated with the risk of rejection within the first month post-transplant (AUROC 0.77,  $p=0.002$ ), but no episodes of infection. On multivariate logistic regression, IFN $\gamma \geq 4.49$  IU/mL (OR 4.75) may be an independent predictor of rejection ( $p=0.05$ ).

**Conclusion:** Low IFN $\gamma$  suggesting over-suppression is associated with infections, while high IFN $\gamma$  indicating under-suppression is associated with rejection. This assay offers the potential to allow individualisation and optimisation of immunosuppression that could fundamentally alter the way patients are managed following transplantation.

## Introduction

Immunosuppression is necessary to encourage long-term engraftment and prevent clinical complications such as allograft rejection following liver transplantation. Clinicians rely on liver biochemistry and drug levels of the commonly used calcineurin inhibitors (CNI) cyclosporin and tacrolimus to guide immunotherapy. However, drug monitoring is an inadequate indicator of immune response (1) and correlates poorly with clinical outcomes. Unfortunately without readily available alternatives, therapeutic drug levels are still typically used to help guide drug dosing. This approach is crude and predisposes patients to risks of over immune suppression that may lead to infection, drug side effects, increased drug costs and increased risk of malignancy; and under immune suppression which leaves patients vulnerable to rejection and graft dysfunction. Indeed, the first indication a patient is either over or under immunosuppressed is often the development of clinical events.

Tailored therapy for each individual patient based on a functional measure of their individual net immune response would be preferable (2), and could reduce clinical complications, improve graft and patient survival while offering substantial cost savings. QuantiFERON-Monitor (QFM, Qiagen, The Netherlands) is a novel immune function biomarker based on the same laboratory platform as the readily used QFN-gold assay (Qiagen, The Netherlands), with potential for next-day results. The QFM assay involves dual stimulation of both the innate and adaptive immune stimulants and provides an objective but non-specific biomarker of immune function. A pilot cross-sectional study showed lower QFM immediately post-transplant, with a recovery as time progresses and immunosuppression withdrawn (3). We present a blinded, observational study that represents the first clinical investigation of QFM in a post-transplant population in order to establish whether it could be used to identify which patients are receiving too much or too little immunosuppression based on the risk of subsequent clinical events of infection and rejection.

## Methods

Adult patients on our centre's liver transplant waiting list were recruited pre-transplant between June 2012 and December 2013 and followed for 12 months post-transplant. Blood samples were collected during a recipient's regular venepuncture pre-transplant, and on post-transplant days (D) 1, 3 and 5, along with week (W) 1 and 2, then monthly (M) from month 1 to 6. Patients who were unable or unwilling to provide informed consent were excluded.

As per manufacturer's guidelines, whole blood was stimulated with QFM, which contains the immune ligands anti-CD3 and R848 (3). Stimulated blood was incubated overnight at 37°C and plasma harvested after centrifugation. Enzyme-linked immunosorbent assay (ELISA) was performed to quantify IFN $\gamma$  output. Clinicians treating the patients were blinded to QFM, whilst laboratory staff performing ELISA were blinded to clinical events.

Primary outcomes were defined as treated and biopsy proven acute rejection (tBPAR) or infection as per pre-defined criteria of 'probable' or 'definite' infection adjusted from the international sepsis forum consensus conference on '*Definitions of Infection in the Intensive Care Unit*' (4). Cytomegalovirus reactivation without disease was not defined as an infection. For a diagnosis of tBPAR, histology had to be confirmed by a specialist liver transplant pathologist and treatment commenced by the transplant physician caring for the patient who was blinded to QFM results. Secondary outcomes included presence of opportunistic infection confirmed by an independent infectious diseases physician, and graft failure or death. QFM results were excluded if the patient had a clinical event within one week prior to their blood test, due to the likely confounding effect that inflammation could have on IFN $\gamma$  production

The Mann-Whitney and Kruskal-Wallis tests were used for nonparametric analysis to detect differences between patient groups. Chi-squared was used to compare nominal variables. Area under the receiver operator curves (AUROC) were used to establish ideal sensitivities and specificities. Clinical (non-QFM) variables that showed potential predictive capacity of 15% ( $p < 0.15\%$ ) were entered into a multivariate logistic regression model. Data was analysed using GraphPad Prism for Mac version 6.0 (GraphPad, California, USA) and Stata SE for

Mac version 12 (Statacorp, Texas, USA). No organs were donated from prisoners or institutionalised persons. The study was approved by the Austin Human Research Ethics Committee.

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## Results

Of 84 consecutive adult liver transplant operations, 75 recipients were recruited prior to, and followed longitudinally after liver transplantation. Reasons for exclusion included five patients with fulminant liver failure who were unable to consent for the study, and a further 4 patients who declined to participate (Figure 1). The majority of recipients were male (n=49, 65.3%) and born in Australia (n=60, 80.0%). The median age at transplantation was 53 years old (range 19-70). Severity of liver disease at time of transplantation was determined with the median Model for End-stage Liver Disease (MELD) score of 20 and Child-Pugh score of 10. Almost all patients had cirrhosis (n=72, 96.0%), with common aetiologies of liver disease outlined in Table 1. Thirty-two percent (n=24) were transplanted for a primary indication of hepatocellular carcinoma (HCC).

The median donor age was 53 years old and the cold ischaemic time 6.1 hours. The majority of grafts were donated from brain dead donors (n=67, 89.3%), with only 8 organs donated after cardiac death (DCD, n=8, 10.7%). Only 2 patients (2.67%) had split transplants, with the other half of the donor organ going to a secondary recipient (a child or an interstate recipient). Neither cold ischaemic time or donor age was associated with clinical events.

Immunosuppression consisted of standard doses of steroids, a CNI and azathioprine or mycophenolate mofetil (MMF). Initial steroid therapy was with a weaning dose of intravenous methylprednisolone over 5 days, following which oral prednisolone was commenced. This was weaned as per physician preference over the first 3-6 months (dotted line/shaded area, Figure 2). Approximately half the patients (n=36, 48.0%) were given Basiliximab (anti-IL-2) as a renal preserving measure on days 1 and 4 in a CNI-sparing protocol.

The median pre-transplant QFM was 121IU/mL. This fell to a nadir of 0.20IU/mL at day 3, after which it gradually increased over the first month post-transplant. As time progressed and immunosuppression reduced, QFM gradually increased before plateauing from month 4 onwards (Figure 3).

## Clinical Events

A majority of recipients (55/75, 73.3%) experienced a total of 89 confirmed clinical events (infection, tBPAR and death). The first documented event was infection in 31 (41.3%) and tBPAR in 23 (30.7%) patients. The median time to tBPAR was 15.0 days, compared with 31.5 days for infection. One patient died early post-transplant with no preceding infection or rejection. The most common time for tBPAR was between W1 and M1 post-transplant, when 17/29 (58.6%) tBPAR episodes were diagnosed, with a median rejection activity index (RAI) score of 6 (range 4-9).

Infections followed a more even distribution until M6 post-transplant, when the infection rate fell sharply (Table 2). Of the infection episodes, eleven were recorded as opportunistic infections that occurred a median 18 days post-transplant (range 10-353 days).

Aetiology of underlying liver disease was not associated with the recipient's first clinical event ( $p=0.96$ ). When examining the commonest indication for transplant, patients with hepatitis C virus (HCV,  $n=33$ ) did not have more frequent rejection or infection compared with other aetiologies ( $p=0.47$ ). The presence of hepatocellular carcinoma (HCC) did not impact infection or rejection outcomes ( $p=0.35$ ).

Three deaths occurred during the study period, resulting in a 12-month survival of 96.0%. Causes of death included cardiac failure following a cardiovascular event suffered at time of transplant, toxic epidermal necrolysis as a result of an antibiotic drug reaction (trimethoprim-sulfamethoxazole), and sepsis resulting in multi-organ failure late post-transplant. Neither pre-transplant nor post-transplant QFM were associated with mortality. No recipient required re-transplantation within 12 months due to graft failure.

Because the largest proportion of events ( $n=34$ , 38.2%), including the majority of tBPAR occurred between W1-M1, we focused on the W1 QFM in otherwise clinically stable patients, and its ability to predict early clinical events.

### **Early Clinical Events (W1-M1)**

W1 QFM was available in  $n=60$  ( $n=12$  had experienced an infection and  $n=1$  rejection in the week prior and were excluded from the W1 analysis;  $n=2$  had venepuncture failure) (Figure 1). There was no difference in age, gender, pre-

transplant MELD, pre-transplant child-pugh score, donor age or choice of immunosuppression between patients with an early clinical event before W1, and those who remained well at W1 ( $p>0.05$  for each). In the 60 clinically stable recipients, the median W1 QFM was 1.66IU/mL. Approximately half this cohort experienced a primary outcome before M1 ( $n=28$ , 46.7%), with an even split between tBPAR ( $n=14$ ) and infection ( $n=14$ ) (Table 1). One patient who experienced both infection and tBPAR within M1 was classified as infection, based on it being the earlier event. One patient who died between W1-M1 had a W1 QFM of 2.44IU/mL, but was severely compromised after an intraoperative cardiac complication.

The median QFM from W1 varied significantly amongst patients who remained well ( $n=31$ , 1.04 IU/mL), tBPAR (3.96 IU/mL) and infection (0.81 IU/ml) ( $p=0.001$ ) (Figure 1). The W1 QFM did not vary according to the immunosuppression used (azathioprine versus MMF, or cyclosporin versus tacrolimus).

The emergence of tBPAR was not associated with the white cell count (WCC) ( $p=0.22$ ), neutrophil count ( $p=0.42$ ), nor CNI drug levels for tacrolimus ( $p=0.40$ ) or cyclosporin ( $p=0.64$ ). Likewise, no non-QFM factors were associated with infection to M1: WCC ( $p=0.98$ ), neutrophil count ( $p=0.66$ ), diabetes ( $p=0.10$ ), eGFR $<50$  ( $p=0.73$ ), tacrolimus level ( $p=0.33$ ) and cyclosporin level ( $p=0.29$ ).

Low W1 QFM was significantly associated with the risk of infection (Figure 4, AUROC 0.74,  $p=0.008$ ). A QFM  $\leq 1.30$ IU/mL (10/27, LR+ 1.93, sensitivity 71.4%, specificity 63.0%, PPV 0.37, NPV 0.88,  $p=0.02$ ) was the best discriminator, defining the best clinical threshold for infection, while displaying minimal risk of rejection to M1. Nearly half the entire cohort (27/60, 45.0%) expressed a QFM  $\leq 1.30$ IU/mL (Figure 6 - bottom shaded area), but only 1/14 episodes of tBPAR occurred below this cut-off, with a value of  $>1.30$ IU/mL therefore associated with a 92.9% sensitivity and 52.2% specificity for tBPAR (LR- 0.14).

Many of the 14 infections (listed in Table 3) were opportunistic ( $n=8$ , 57.1%, marked as stars in Figure 6). Three of the five lowest W1 QFM results recorded in any patient, were in those who later developed severe opportunistic infections: cryptococcal meningitis, herpes simplex hepatitis and fungal peritonitis. This correlated with a significant difference between patients who

had an opportunistic infection, and patients with no infection (AUROC 0.72,  $p < 0.05$ ). A lower optimal W1 QFM cut-off  $< 0.33 \text{ IU/mL}$  was associated with a LR+ 7.67 (sensitivity 50%, specificity 93.5%) for opportunistic infections, although only 8/60 (13.3%) of all recipients had a QFM under this very low (severely immunosuppressed) cut-off.

An elevated W1 QFM (suggestive of insufficient immunosuppression) significantly correlated with the risk of rejection to M1 (Figure 5, AUROC 0.77,  $p = 0.002$ ). A QFM  $\geq 4.49 \text{ IU/mL}$  had a positive likelihood ratio for tBPAR of 3.3 (7/14, sensitivity 50.0%, specificity 84.5%, PPV 0.50, NPV 0.85,  $p = 0.01$ ). This would classify 14/60 (23.3%) of recipients as 'at risk' of rejection (Figure 6 – top shaded area). No patients with a W1 QFM  $\geq 4.49 \text{ IU/mL}$  experienced an infection to M1, and a QFM  $< 4.49 \text{ IU/mL}$  was therefore associated with a 100% sensitivity and 28.3% specificity for infection.

Employing a lower QFM cut-off of  $\geq 2.61 \text{ IU/mL}$  would still offer a LR+ of 2.3 and have improved sensitivity of 64.3%, but reduced specificity of 71.7% (PPV 0.41, NPV 0.87). This would classify more patients, 22/60 (36.7%), as under-suppressed and 'at risk' of rejection.

### ***Factors predictive for rejection***

A high W1 QFM above the selected cut-off ( $\geq 4.49 \text{ IU/mL}$ ) had a significant OR of 5.57 for tBPAR to M1 ( $p = 0.01$ ). On univariate analysis, Basiliximab was the only protective factor against tBPAR with an odds ratio (OR) of 0.25 ( $p = 0.03$ ) (Table 4). No other variable, including age, gender, HCV, HCC, cold-ischaemia time, donor age, pre-transplant MELD or pre-transplant Child-Pugh scores were associated with risk of early rejection. All variables that showed potential predictive capacity of 15% ( $p < 0.15$ ) were entered into a multivariate logistic regression model. In this model, age (OR 0.92,  $p = 0.02$ ) and a high QFM (OR 4.75,  $p = 0.05$ ) were potential independent factors of rejection (Table 5).

## Discussion

The balance of immunosuppression following liver transplantation is precarious, and the risk of clinical complications is subsequently high. Drug monitoring is a poor indicator of immune suppression (1) and the U.S. Food and Drug Administration (FDA) has reclassified assays for measuring tacrolimus and cyclosporin blood levels indicating that no suitable therapeutic ranges exist and these tests may not be used alone to adjust drug dosing (5). Without suitable alternatives, therapeutic drug levels are still employed to guide therapy in a substandard approach that predisposes patients to risks of over immune suppression that can lead to infections, drug side effects, increased drug costs and malignancy. Conversely, the risk of under immune suppression leaves patients vulnerable to rejection and graft dysfunction with rejection rates following liver transplant still ranging between 30-40% (6-8). It is estimated that 40-70% of all mortality following transplantation is potentially related to immunosuppression or immunosuppressive medications (9, 10). Therefore, an objective measure of a recipient's immune function is desperately needed.

QFM incorporates both an innate and adaptive stimulant, which offers an objective, albeit non-specific overview of an individual's ex-vivo immune response. The assay fulfils many characteristics believed to be essential for an immune function biomarker. It is accessible (based on QFN-gold, an assay already in widespread use), rapid and requires minimal laboratory processing. Furthermore, the level of interferon gamma production after stimulation with the combination of these innate and adaptive immune stimulants has been shown to correlate with immunosuppression levels in a pilot cross-sectional study (11). This current study represents the first use of the assay in a clinical cohort of organ recipients for which it was intended, and demonstrates clear associations between QFM and clinical events.

A low W1 QFM  $\leq 1.30$  IU/mL was associated with significant risk of infection (LR+1.93,  $p=0.02$ ). QFM accurately classifies these recipients as over-suppressed, with a significant risk of infection and very minimal chance of rejection. These patients (including those who had remained well despite having low QFM) could conceivably have their immunosuppression reduced. Importantly, 45% of the

entire cohort had a QFM below this cut-off. This would offer a large proportion of organ recipients the opportunity to reduce their immunosuppression, thus offering reduced drug costs and side-effects which are particularly relevant given the association between immunosuppressive agents and morbidity and mortality following liver transplantation. Not only was low QFM associated with infections, but the magnitude of immunodeficiency correlated with more severe opportunistic infections. Conversely, an elevated W1 QFM  $\geq 4.49$  IU/mL was significantly associated with early rejection (LR+ 3.3,  $p=0.01$ ), and in a multivariate model, a high QFM was a potential independent predictor of early rejection with an odds ratio of 4.75, but  $p=0.05$  (Table 5). Perhaps more importantly, no patient with a W1 QFM  $\geq 4.49$  IU/mL experienced an infection within the first month post-transplant. These patients (which represent 23% of the cohort) could have their immunosuppression increased in an attempt to ameliorate the risk of tBPAR, with minimal risk of infection.

We focused on the W1 QFM blood test for several reasons. Firstly, the greatest concentration of clinical events occurred between W1-M1 post-transplant (54.8% of all rejection, 28.1% of infections), after which events became less frequent (Table 2). Secondly, because the W1 blood test occurs before the majority of clinical events it may offer the best potential predictive capacity, yet still allow sufficient time for modifications in immunosuppression to impact future clinical outcomes. Thirdly, the early post-transplant period is particularly important in establishing allorecognition responses. Land's injury hypothesis assumes that innate immune triggers that are initiated and propagated at time of transplantation factor into the creation of allo-responsive T-cells that prevent the development of tolerance (12). As such, the week 1 time-point has been identified as critical in other ex-vivo laboratory studies in regards to detecting rejection (13).

Clinical events were common in this transplant cohort, with nearly three quarters of patients experiencing an outcome over 12 months. The tBPAR rate of 38.7% is notably high, but similar to that documented in other published studies (6-8). However, some of these studies are from the early 2000s and rates are likely to vary depending on a transplant unit's preference for type and dosing of immunosuppression. The high frequency of infection and rejection serves to

highlight the potential value of QFM. Based on selected cut-offs, 68.3% of liver transplant recipients appear to be receiving either too much (45.0%) or too little immunosuppression (23.3%), thus demonstrating the inherent difficulties in balancing immunosuppression following transplantation. However, not all clinical outcomes are likely to be preventable, just as not all patients who are over or under-suppressed would be expected to have infection or rejection.

An alternative immune function biomarker (ImmuKNOW, Cylex Ltd, USA) has been approved by the FDA for monitoring immune function following transplantation. Studies in liver transplant recipients have reported contradictory results for ImmuKnow in predicting acute rejection and infection (14-21). Most of these studies are retrospective, have limited follow-up, are heterogeneous in study design, and often include multiple solid organ transplants in the analysis, despite immunosuppression protocols and clinical event profiles differing substantially amongst different solid organ transplant populations (22). To coincide with the multiple studies, there have been two somewhat contradictory meta-analyses (23, 24), and the assay has failed to achieve widespread acceptance as standard of care internationally. A possible explanation for the inconsistencies may be that it relies on T-cell stimulation with PHA mitogen, which is an adaptive immune system stimulant. However, current evidence favours a combined impact of both the innate and adaptive immune responses in rejection and allorecognition (12, 25). This is a strength of the QFM assay, which measures immune response following stimulation of both arms of the immune system.

However, because QFM relies on stimulation of the innate and adaptive immune systems, the presence of a clinical event is likely to act as a confounder for the assay result. In the setting of a clinical event such as infection or rejection, it is likely that QFM would be measuring a component of the already present inflammatory response, rather than a recipient's potential immune function. If a patient had a clinical event in the week prior to a QFM blood test, that result was excluded from analysis for the risk of subsequent outcomes. Similarly, in clinical practice QFM is unlikely to be applicable if a clinical event has recently occurred. Aetiology of underlying liver disease was not associated with clinical outcomes, and has not been shown to impact QFM. This has particular importance in

regards to HCV infection. QFM contains the innate immune stimulant R848, a ligand for Toll-like receptors (TLR) 7 and 8. Although much remains unknown about these TLRs, current evidence suggests a specific role in recognition of single stranded ribonucleic acid (ssRNA) viruses (26). HCV, a ssRNA virus, has been the most common recent indication for liver transplantation in western nations. Reassuringly, there appears limited evidence that the use of QFM is compromised in the presence of HCV infection.

Patients following liver transplant often have mild abnormalities of liver biochemistry. Early liver biopsy changes, although often consistent with mild rejection, can also represent post-transplant ischaemia related cholestasis. Many patients may not therefore receive treatment for acute cellular rejection on biopsy as they improve spontaneously. As such, we employed the strict definition requiring not only consistent histology, but also treatment of rejection (tBPAR) as is standard in high quality studies of liver transplantation. This may risk some rejection being underreported as it relies on physician preference to treat, however the fact that patients who are not treated must improve spontaneously suggests that their rejection, if truly present, would be of limited clinical consequence.

Infections are often difficult to define, and in post-transplant patients, early empirical treatment is common. We used pre-defined criteria of 'probable' or 'definite' infection adjusted from the international sepsis forum consensus conference on '*Definitions of Infection in the Intensive Care Unit*' (4). Other strengths of this study are that patients were prospectively recruited and the clinicians blinded to results.

However, there were difficulties when performing the first translational clinical study employing a novel assay such as QFM in a transplant population. With only a cross-sectional pilot study (3) to act as guidance, there were no pre-defined thresholds for assay results. As such, the selected cut-offs were determined in retrospect. Furthermore, as each transplant unit employs different immunosuppressive regimens the optimal QFM range identified in this study of 1.30-4.49IU/mL would need further validation in multicentre studies. Future studies with longer term follow-up will also be needed to identify hard end-points such as graft failure, development of renal failure and death that are

unable to be accurately explored in this 12 month study. Clearly, the findings of this study may only be applied to a liver transplant cohort, and may not be applicable to other solid organ transplant populations.

These studies would also be needed to answer remaining questions that were out of scope of this current study. With the multiple medications and doses involved at each separate time point, and the impact that time itself is likely to have on post-transplant immune responses, the relative impact of each individual immunosuppressant on QFM has been difficult to establish. Interventional studies are likely to be difficult without identifying how much of a change in immunosuppression is required to reduce or increase QFM in order to sufficiently reduce the risk of a clinical event. Accumulated experience will likely be needed in order to reduce clinical outcomes, but also identify the appropriate dose changes to minimize the risk of a rebound effect, which may result in the alternate outcome eventuating. For example, over-treating a patient with a high QFM  $\geq 4.49$  IU/mL and precipitating a subsequent infection. It is also unknown whether repeated measures after a change in immunosuppression may assist in balancing these risks.

Managing patients based on drug levels or physician experience although reasonably effective, still results in significant numbers of clinical events as well as drug related side effects that cause much morbidity and mortality (9, 10). The QFM assay objectively identified 70% of our cohort as being over or under immunosuppressed, with low QFM associated with early infection but not rejection, and high QFM associated with rejection but minimal risk of infection. Although multi-centre validation cohorts along with randomized interventional trials are needed, the potential to individualise and therefore optimize immunosuppression based on an objective immune function biomarker may fundamentally alter the way patients are managed following liver transplantation.

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Figure Legends:

**Figure 1 - Patient flow chart**

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**Figure 2 – Median immunosuppression drug levels and prednisolone dosage**

*Because of the relative potency of tacrolimus versus cyclosporin, in order to directly compare tacrolimus and cyclosporin, the cyclosporin level is presented as cyclosporin level (taken as C2 level 2 hours after dosing) divided by 100. Shaded area refers to oral prednisolone dose (mg). Standard doses of weaning intravenous methylprednisolone were used for the first 5 days post-transplant.*

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**Figure 3 - Median QFM in all transplant recipients (n=75) expressed on a logarithmic scale**

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**Figure 4 - Sensitivity and Specificity of W1 QFM for risk of infection to M1**

*Note that as QFM increases, the specificity of QFM for infection reduces while sensitivity increases. This is opposite to tBPAR (Figure 5). Vertical dotted line represents the cut-off of 1.31 IU/mL.*

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**Figure 5 - Sensitivity and specificity for W1 QFM for diagnosing tBPAR to M1**

*The vertical dotted line represents the cut-off of 4.49 IU/mL. This is further along the x-axis than the intersection point. This cut-off allows improved specificity at a cost to sensitivity for future rejection events to M1.*

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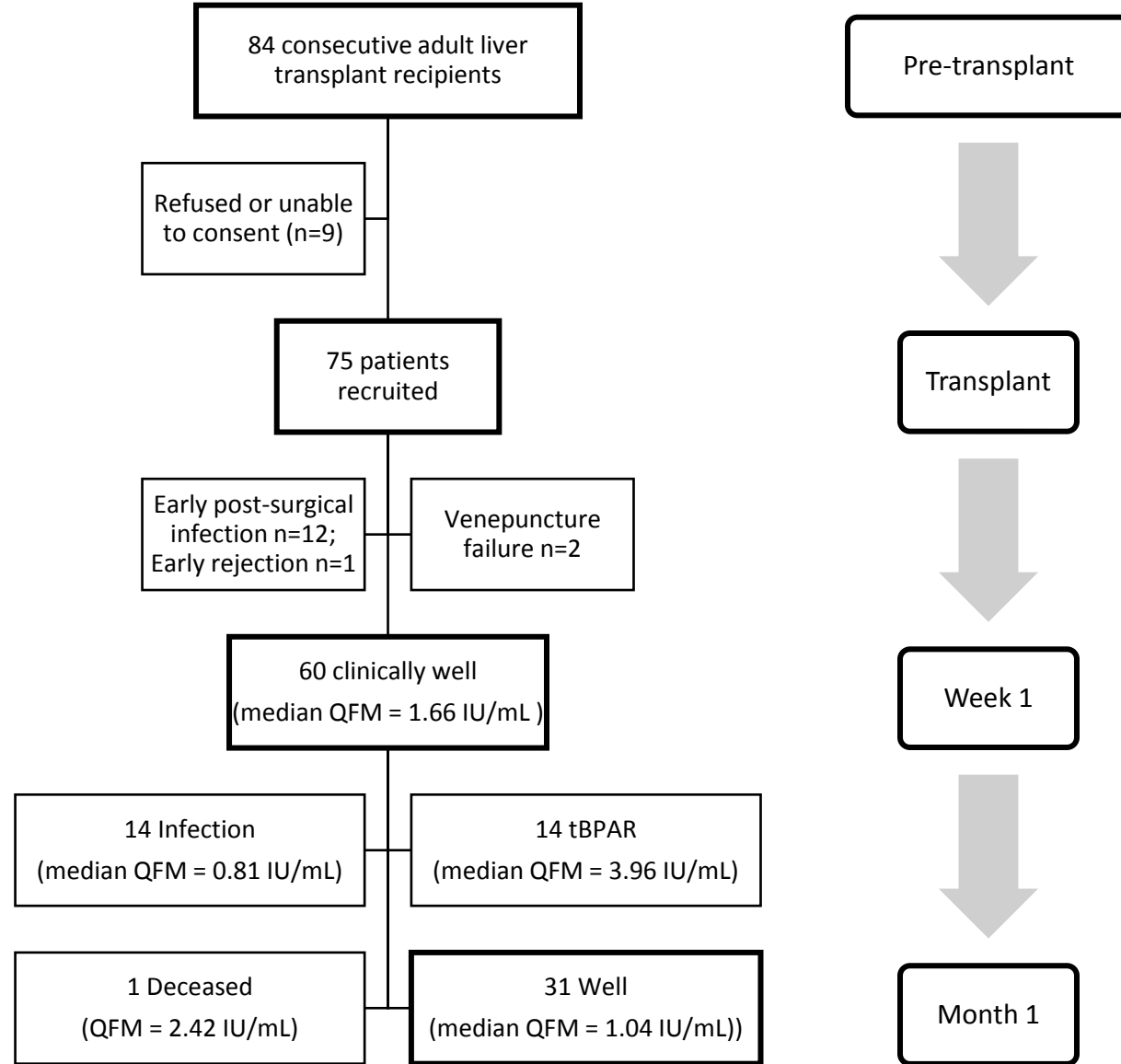
**Figure 6 - Week 1 QFM and Clinical Events occurring to 1 Month Post-transplant**

\* = opportunistic infections.

Only includes patients without a prior event at W1 (valid W1 QFM, n=60.). Top shaded area (23.3% of cohort) shows patients felt to be under-suppressed (should increase immunosuppression), while bottom shaded area (45.0% of cohort) shows patients believed to be over-suppressed (should reduce immunosuppression) according to QFM measurements. Note that **no** infection events occurred in the top shaded area, and only **one** rejection event was documented in the bottom shaded area.

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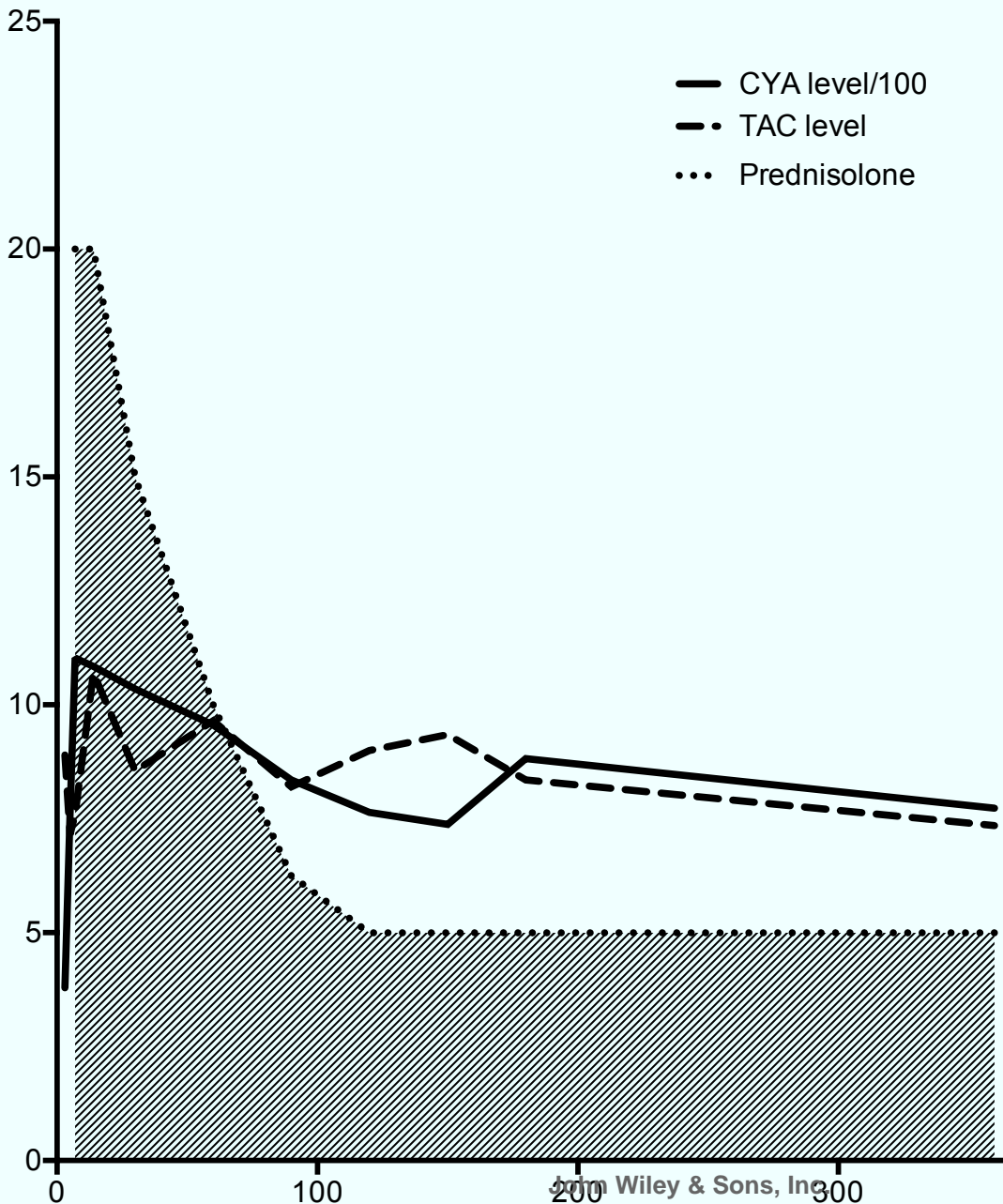
### Liver Transplantation



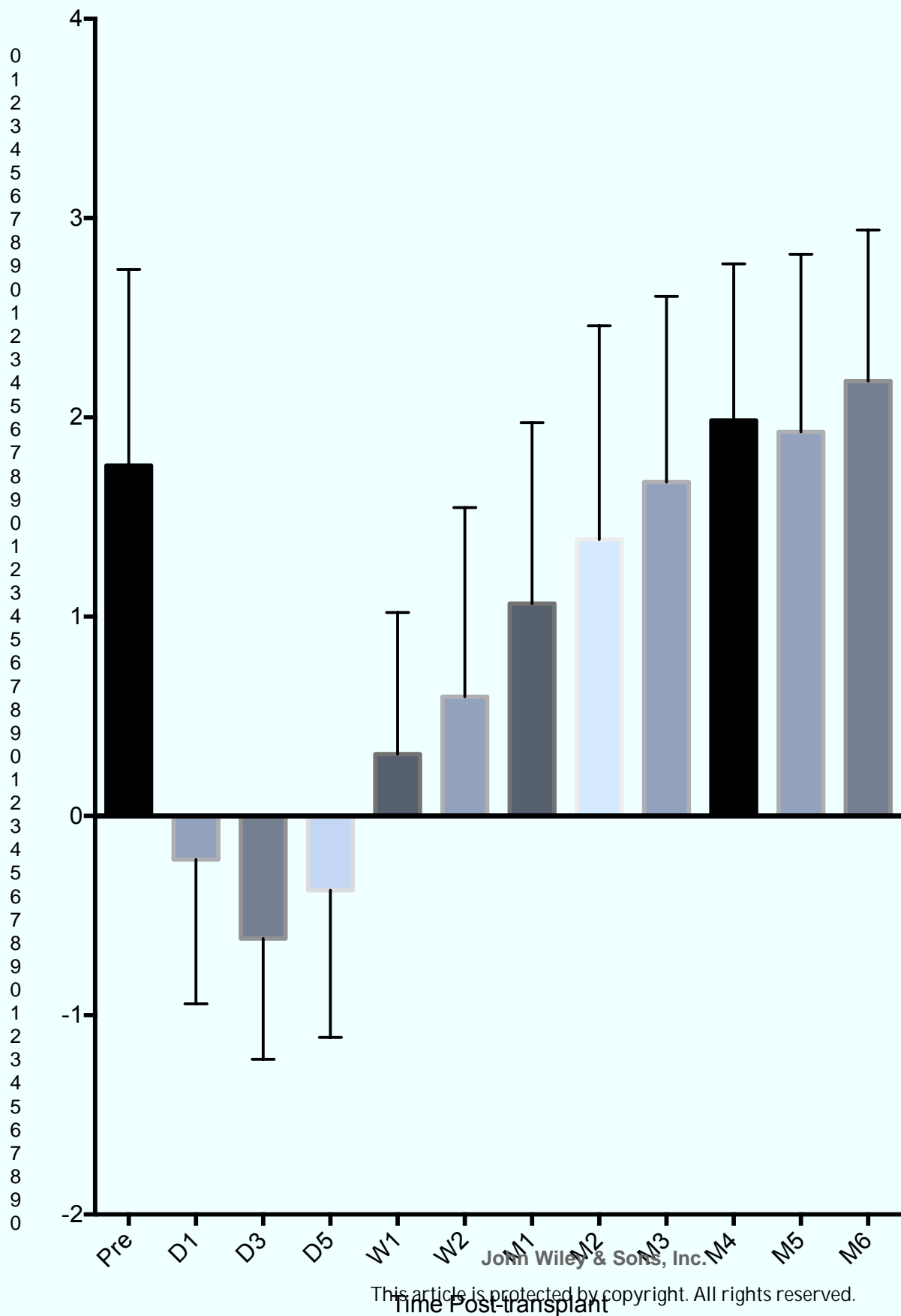
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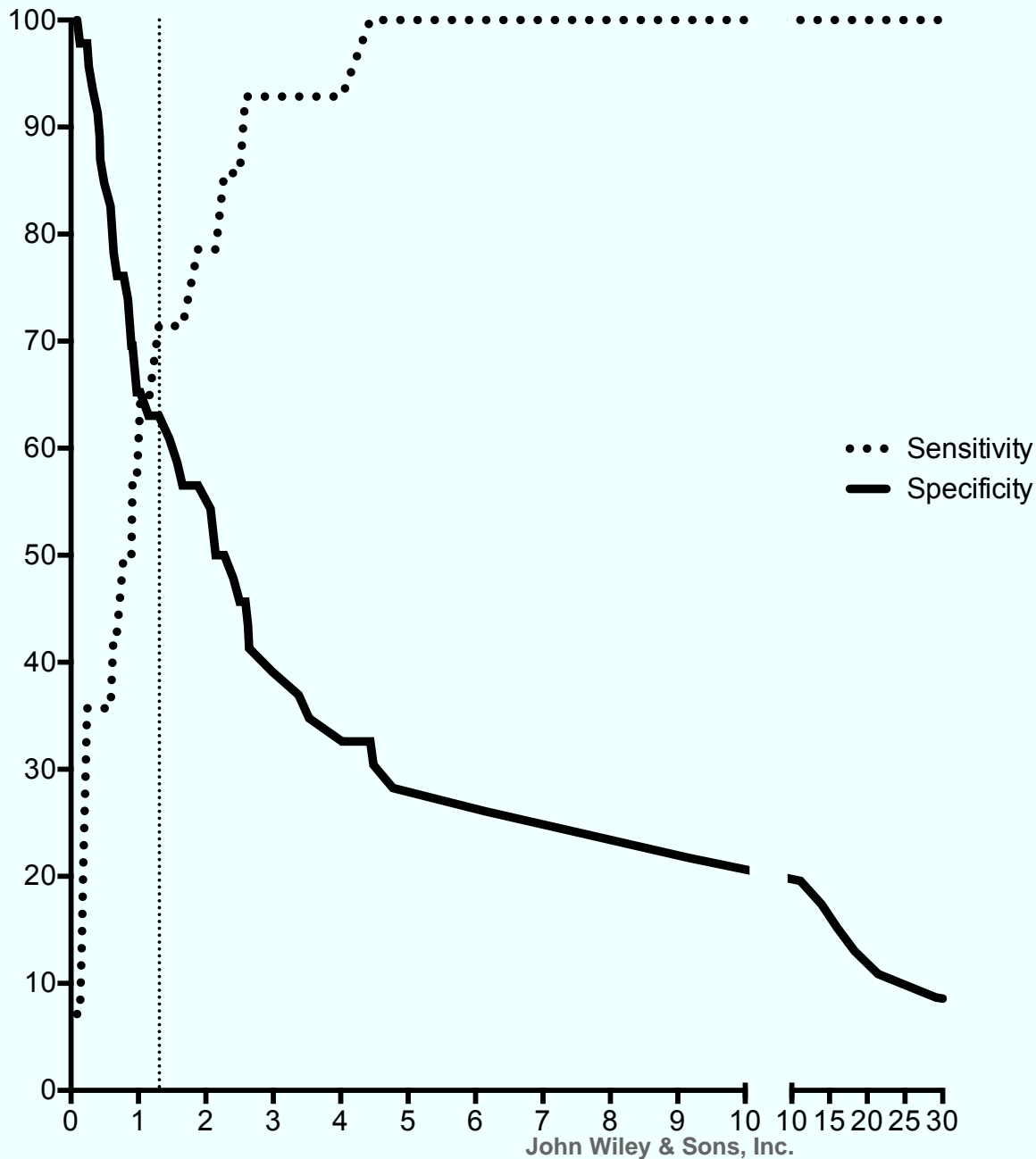


### QFM in all patients (logarithmic scale)



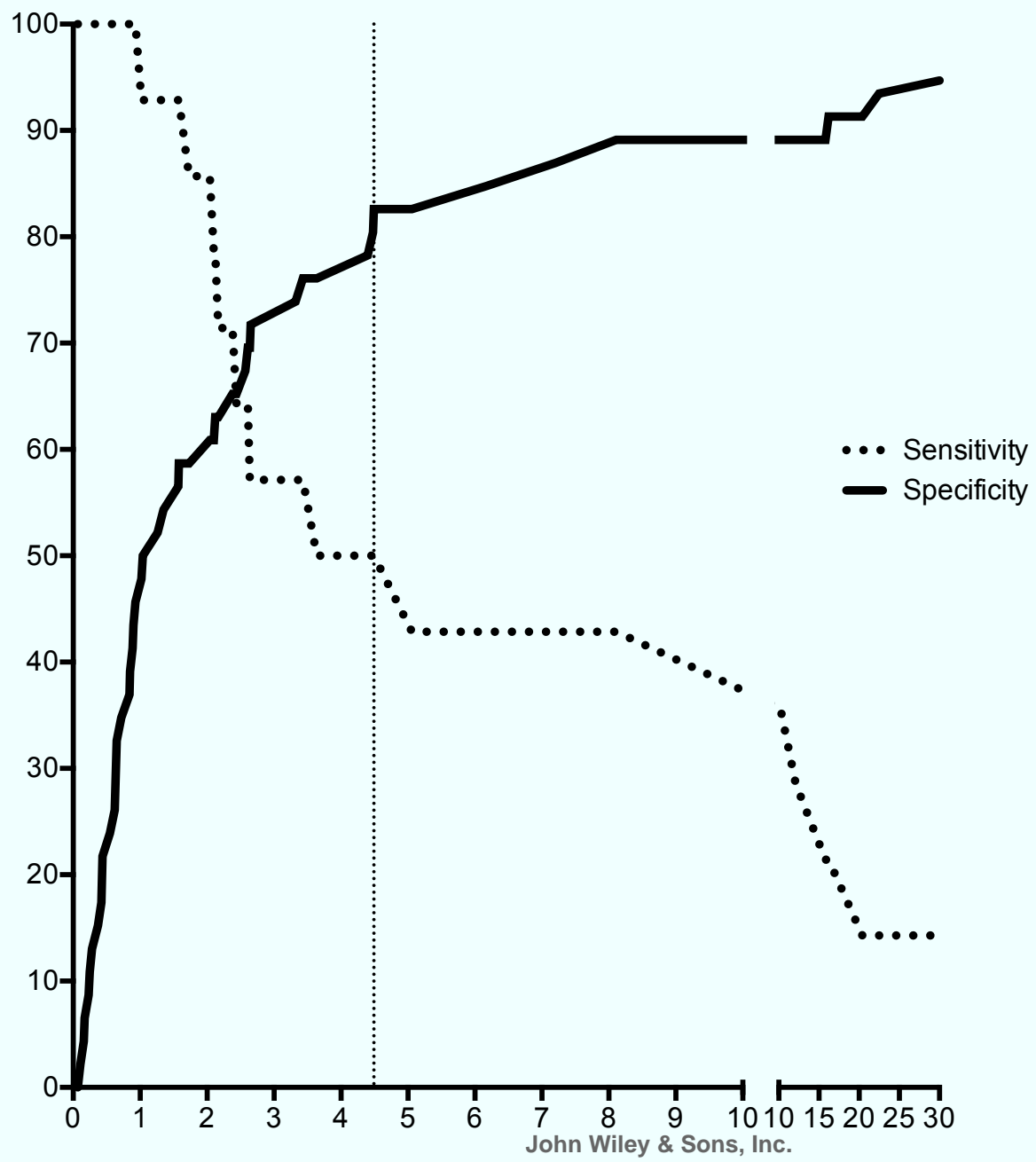
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### W1 QFM and Clinical Events to M1

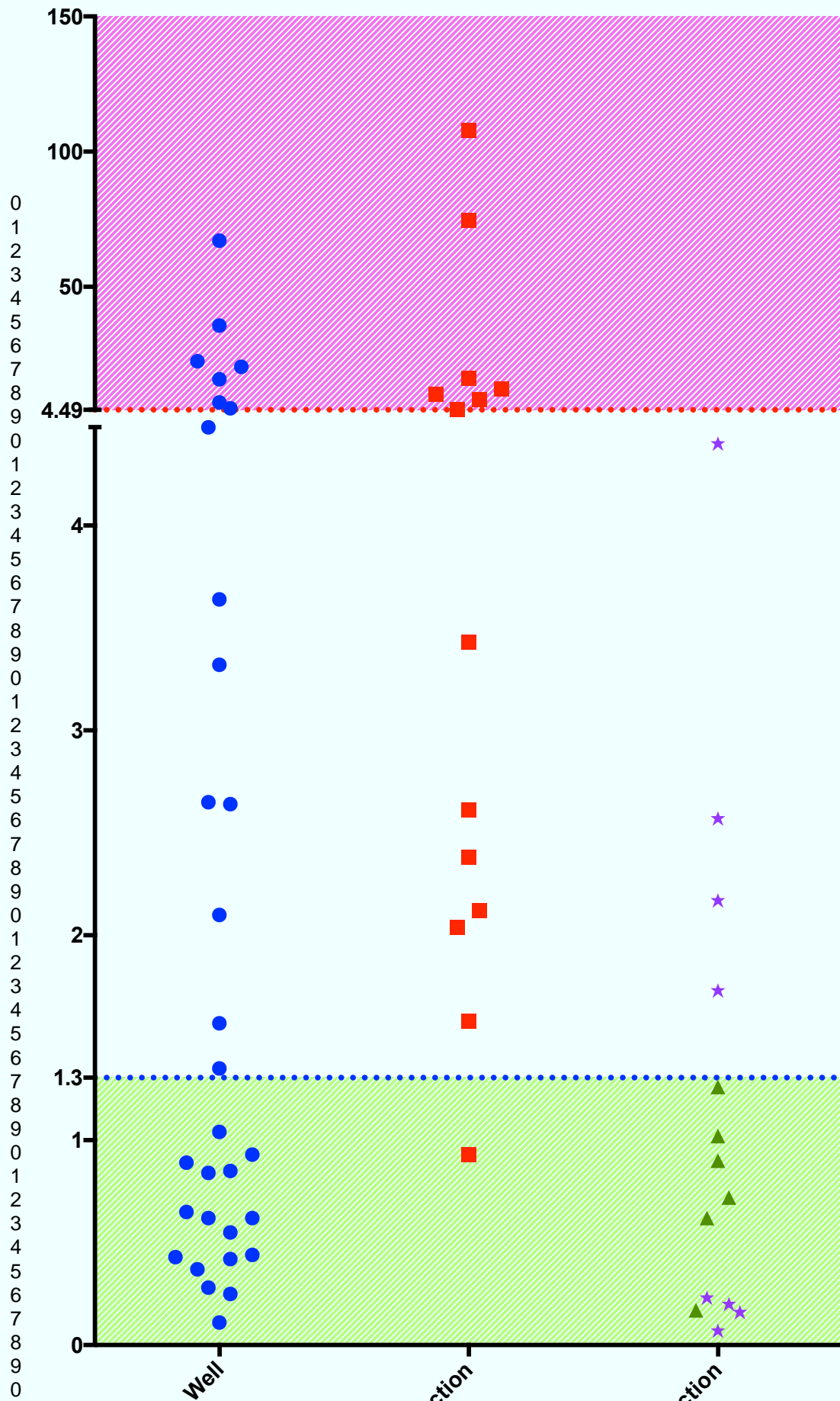


Table 1 - Median characteristics of all patients, and with early clinical events

	All Patients	Clinical Events From 1 Week To 1 Month Post-Transplant*			
		Well	tBPAR	Infection	p
Patients	75	31	14	14	
Age	53	52	48	55	0.08
Male	49 (65.3%)	19 (61.3%)	9 (64.3%)	10 (71.4%)	0.81
Cirrhotic	72 (96.0%)	30 (96.8%)	14 (100%)	14 (100%)	0.90
Aetiology of Liver Disease (n)					0.46
HCV	33 (44.0%)	14 (45.2%)	4 (28.6%)	10 (71.4%)	0.07
NASH	8 (10.7%)	5 (16.1%)	1 (7.14%)	0	0.23
PSC	7 (9.33%)	2 (6.45%)	2 (14.3%)	0	0.32
ETOH	7 (9.33%)	2 (6.45%)	3 (21.4%)	2 (14.3%)	0.34
AIH	5 (6.67%)	3 (9.68%)	1 (7.14%)	0	0.49
PBC	4 (5.33%)	1 (3.23%)	1 (7.14%)	0	0.58
Other	11 (14.7%)	4 (12.9%)	2 (14.3%)	2 (14.3%)	0.99
Hepatocellular carcinoma	24 (32.0%)	11 (35.5%)	3 (21.4%)	5 (35.7%)	0.61
Pre-transplant MELD	20	22	16.5	25	0.11
Pre-transplant Child-Pugh	10	11	9.5	10.5	0.71
Donation after cardiac death (DCD)	8 (10.7%)	4 (12.9%)	1 (7.14%)	1 (7.14%)	0.77
Cold-ischaemic time (h)	6.10	6.10	5.65	6.35	0.54
Donor age (median)	53.0	54.0	57.0	54.5	0.94
Pre-transplant QFM (IU/mL)	121.0	123.3	139.5	85.05	0.54
Pre-transplant Vitamin D	72	80	65	46.5	0.60

The table includes patients who had a valid W1 QFM (ie: no clinical complication in the first week post-transplant) (n=59), not including the one patient who died between W1-M1 who is discussed separately.

HCV – Hepatitis C; NASH – Non-alcoholic steatohepatitis; PSC – Primary sclerosing cholangitis; ETOH – Alcoholic liver disease; AIH – Autoimmune hepatitis; PBC – Primary biliary cholangitis.

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Table 2 – Timing of All Clinical Events

	D1-W1	W1-M1	M1-M6	M6-M12	Total
tBPAR	1	17	8	3	29
Infection	12	16	22	7	57
Deaths	0	1	1	1	3

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**Table 3 – Infections occurring between W1-M1**

	Type of Infection	Organism grown
Peritonitis	6	n=2 fungal
Ophthalmitis	1	Fungal
Pneumonia	2	
Multiorgan	1	CMV
Cellulitis	1	Pseudomonas
Genital infection	1	HSV
Hepatitis	1	HSV
Shingles	1	HSV

Table 4 - Univariate analysis of possible factors affecting tBPAR to M1

	OR	p	95% CI
Age	0.96	0.11	0.92-1.01
Male	0.96	0.95	0.31-2.99
HCV	0.63	0.41	0.20-1.92
HCC	0.58	0.40	0.17-2.03
Cold-ischaemic time	0.84	0.32	0.59-1.18
Donor age	1.00	0.80	0.97-1.04
MELD	0.94	0.13	0.87-1.02
Child-Pugh	0.92	0.51	0.71-1.18
Basiliximab	0.25	0.03	0.07-0.86
QFM $\geq$ 4.49 IU/mL	5.57	0.01	1.49-20.9

Table 5 - Multivariate logistic regression analysis of factors affecting rejection between W1-M1

	Odds Ratio	p	95% CI
Age	0.92	0.02	0.86-0.99
Pre-transplant MELD	0.90	0.17	0.78-1.04
Basilixmab	0.32	0.27	0.04-2.43
QFM $\geq$ 4.49 IU/mL	4.75	0.05	0.99-22.8

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