Low Pretransplantation Mannose-Binding Lectin Levels Predict Superior Patient and Graft Survival after Simultaneous Pancreas-Kidney Transplantation

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ABSTRACT
Simultaneous pancreas-kidney transplantation (SPKT) is the treatment of choice for patients with type 1 diabetes and renal failure. However, this procedure is characterized by a high rate of postoperative infections, acute rejection episodes, and cardiovascular mortality. The lectin pathway of complement activation contributes to cardiovascular disease in diabetes and may play an important role in inflammatory damage after organ transplantation. This study therefore sought to determine how mannose-binding lectin (MBL), a major recognition molecule of the lectin pathway of complement activation, influences outcome after SPKT. MBL serum levels were determined in 99 and MBL genotypes in 97 consecutive patients who received an SPKT from 1990 through 2000 and related to patient and graft survival. At 12 yr, cumulative death-censored kidney graft survival was 87.5% in patients with an MBL level <400 ng/ml and 74.8% in the group with MBL levels >400 ng/ml (P = 0.021). Pancreas graft survival was significantly better in patients with low MBL levels (P = 0.016). MBL levels >400 ng/ml were associated with a hazard ratio of 6.28 for patient death (95% confidence interval 1.8 to 20.3; P = 0.003). Accordingly, survival was significantly better in recipients with MBL gene polymorphisms associated with low MBL levels. These findings identify MBL as a potential risk factor for graft and patient survival in SPKT. It is hypothesized that MBL contributes to the pathogenesis of inflammation-induced vascular damage both in the transplanted organs and in the recipient’s native blood vessels.


Simultaneous pancreas-kidney transplantation (SPKT) is the preferred treatment option for patients with long standing type 1 diabetes and end-stage renal failure. The major arguments favoring SPKT in these patients rather than renal transplantation alone include improved quality of life, prevention of recurrent diabetic nephropathy, and stabilization of diabetic neuropathy and retinopathy. Recent studies demonstrated that SPKT, compared with kidney transplantation alone, leads to improved allograft survival1 and improved patient survival.2,3 Despite these benefits, mortality after SPKT transplantation remains high, with 10-yr patient survival rates of <70%.2,4

The complement system contributes to tissue damage at various stages of the transplantation process. An important role in ischemia/reperfusion injury and acute rejection has been demonstrated in various animal models.5,6 Recently, the F/F and F/S donor allotypes of the C3 complement molecule have been associated with better long-term outcome after kidney transplantation.7

Mannose-binding lectin (MBL) is the major rec-
In both patients with type 1 and type 2 diabetes.15–17
an increased risk for vascular disease and diabetic nephropathy
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first exon of the MBL gene in both a control
correlates with the presence of SNP in the
between high and low MBL levels. This cutoff
transplantation was 1053 ng/L. The median
The mean MBL concentration in the 99
RESULTS
mortality.
appears in situations of impaired adaptive immunity, such as early childhood or
in host defense, wild-type MBL binds to carbohydrate
and elimination of pathogens. Single-nucleotide polymor-
may also interact with tissue and lead to complement-medi-
comparable in both groups (1.85
Likewise, the number of rejection treatments per patient was
MBL could be a major determinant of
by a high rate of infectious complications,
acutely infectious agents. This is especially apparent in situations
that MBL could be a major determinant of
Many studies showed an association of low serum MBL
levels and MBL SNP with decreased host defense against vari-
by a high rate of infectious complications, acute graft rejection, and cardiovascular
Our group has shown that low pretransplantation MBL lev-
levels are associated with better graft survival after deceased-donor kidney transplanta-
tion.18 In view of the role of MBL in diabetes and transplantation, we hypothesized
Our group has shown that low pretransplantation MBL lev-
levels are associated with better graft survival after deceased-donor kidney transplanta-
population19 and the recipients studied here (Figure 1). The
median MBL concentration in SPKT recipients with only wild-
type MBL alleles (A/A) was 1493 ng/ml (n = 54). In recipients
with the A/O (n = 29) or O/O (n = 4) genotype, the median
MBL concentrations were 245 and 166 ng/ml, respectively. Of
the patients with an MBL level >400 ng/ml, 89.3% had only
wild-type MBL alleles (A/A), whereas 90% of the patients with
an MBL level <400 ng/ml had at least one of the exon 1 MBL
polymorphisms (A/O or O/O). To assess whether pretrans-
plantation MBL levels are representative of the levels after
transplantation, we determined the MBL concentrations 1 yr
after SPKT in 30 patients and compared them with the levels
measured in the pretransplantation sample. We found a high
intraindividual correlation of MBL levels over time (r = 0.87,
P < 0.0001).
Thirty-four (34.3%) SPKT recipients had a low MBL level,
and 65 (65.6%) recipients had a high MBL level. Table 1 shows
the characteristics of the high and low MBL recipients. No
significant difference between both groups concerning demo-
graphic and clinical characteristics including donor and recipient
age, cytomegalovirus status, and gender distribution was noted. Both groups had a comparable proportion of patients
undergoing SPKT before initiation of dialysis treatment. Both
the high- and low-MBL groups had a comparable proportion
of patients receiving triple immunosuppression including myco-
phenolate motefol. The proportion of patients with at least
one significant coronary stenosis was 27.3% in the low-MBL
group and 22.3% in the high-MBL group (P = 0.58). Of note,
there was no difference in the baseline C-reactive protein
(CRP) levels between the two MBL groups. The majority
of patients required treatment for acute rejection, 88.2 and 86.2% in
the low- and high-MBL groups, respectively (P = 0.99).
Likewise, the number of rejection treatments per patient was
comparable in both groups (1.85 versus 1.68; P = 0.49).

Table 1. Characteristics of study population according to MBL levels

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Acceptor MBL Level (ng/ml)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MBL≤400</td>
<td>MBL&gt;400</td>
</tr>
<tr>
<td>n</td>
<td>34</td>
<td>65</td>
</tr>
<tr>
<td>Recipient age (yr; mean ± SD)</td>
<td>39.9 ± 7.8</td>
<td>40.8 ± 6.8</td>
</tr>
<tr>
<td>Female recipient (%)</td>
<td>32.4</td>
<td>38.5</td>
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<tr>
<td>Years diabetes (mean ± SD)</td>
<td>27.1 ± 6.2</td>
<td>26.2 ± 6.3</td>
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<tr>
<td>Active smoking (%)</td>
<td>32.4</td>
<td>19.7</td>
</tr>
<tr>
<td>Significant stenosis in pretransplantation CAG (%)</td>
<td>27.3</td>
<td>22.2</td>
</tr>
<tr>
<td>Baseline CRP (mg/L; mean ± SD)</td>
<td>4.3 ± 6.4</td>
<td>4.45 ± 7.4</td>
</tr>
<tr>
<td>Baseline cholesterol (mmol/L; mean ± SD)</td>
<td>5.11 ± 1.2</td>
<td>5.27 ± 1.3</td>
</tr>
<tr>
<td>Preemptive SPKT (%)</td>
<td>44.1</td>
<td>35.4</td>
</tr>
<tr>
<td>CMV seropositive (%)</td>
<td>41.2</td>
<td>35.9</td>
</tr>
<tr>
<td>Donor age (yr; mean ± SD)</td>
<td>33.0 ± 9.1</td>
<td>29.8 ± 11.8</td>
</tr>
<tr>
<td>Cold ischemia time (h; mean ± SD)</td>
<td>14.8 ± 2.9</td>
<td>15.2 ± 3.9</td>
</tr>
<tr>
<td>Rejection episodes (mean ± SD)</td>
<td>1.85 ± 0.9</td>
<td>1.69 ± 1.13</td>
</tr>
<tr>
<td>HLA DR mismatches (mean ± SD)</td>
<td>1.32 ± 0.64</td>
<td>1.29 ± 0.63</td>
</tr>
<tr>
<td>Mycophenolate (%)</td>
<td>47.1</td>
<td>47.6</td>
</tr>
</tbody>
</table>

*aCAG, coronary angiogram; CMV, cytomegalovirus; CRP, C-reactive protein; MBL, mannos-binding lectin.*

Figure 1. Pretransplantation mannose-binding lectin (MBL) levels stratified according to MBL genotype. The dashed line represents the cutoff level of 400 ng/ml. MBL levels are represented on a log scale.

RESULTS
The mean MBL concentration in the 99 available sera obtained directly before transplantation was 1053 ng/L. The median
centration was 694 ng/ml. A cutoff of 400 ng/ml was used to discriminate between high and low MBL levels. This cutoff
in the first exon of the MBL gene in both a control
Analysis for death-censored graft survival revealed a significant survival advantage for both the renal and the pancreas allografts in favor of the low-MBL recipients. At 12 yr after transplantation, cumulative death-censored pancreas graft survival was 100% in the low-MBL group versus 82% in the high-MBL group ($P = 0.016$ by the log-rank test with grafts lost within 1 wk excluded; Figure 2A). Death-censored renal allograft survival at 12 yr after transplantation was 87.5% in patients with an MBL level $<400$ ng/ml and 74.8% in patients with an MBL level $>400$ ng/ml ($P = 0.021$ by the log-rank test; Figure 2B).

Subsequently, the MBL status was related to patient survival. Twelve years after transplantation, cumulative patient survival was 86.9% in the low-MBL group and 49.1% in the high-MBL group ($P = 0.001$ by the log rank test; Figure 3A). To examine whether the inferior patient survival in high-MBL recipients was a mere consequence of graft loss, we repeated the survival analysis after excluding the patients who lost either the kidney or the pancreas allograft. In the group with functioning allografts, patient survival remained inferior in those with MBL levels $>400$ ng/ml ($P = 0.02$).

To confirm these findings, we also analyzed recipient survival according to the MBL genotype. Superior survival was found in patients with a variant MBL genotype when compared with recipients with only wild-type MBL alleles ($P = 0.026$; Figure 3B).

We analyzed various characteristics in relation to patient survival (Table 2). An MBL level $>400$ ng/ml was associated with a strongly increased mortality (hazard ratio 6.28; 95% confidence interval 1.89 to 20.87; $P = 0.003$). Accordingly, the presence of wild-type MBL alleles was associated with an increased risk for patient death (hazard ratio 3.6; 95% confidence interval 1.22 to 10.6; $P = 0.02$). MBL was also significantly associated with an increased risk for patient death when analyzed as a continuous parameter ($P = 0.013$). MBL remained significantly associated with patient death when entered into a multivariate model adjusted for recipient age, gender, and baseline CRP using the Cox regression method (Table 2).

The reasons for patient death in the high- and low-MBL groups are shown in Table 3. The excess mortality in patients with an MBL level $>400$ ng/ml was explained to a large extent by a higher cardiovascular mortality in this group. No significant difference in infection-related deaths between the low- and high-MBL groups was observed.

**DISCUSSION**

Our study demonstrates superior graft and patient survival after SPKT in recipients with low MBL levels. A high MBL level was associated with an increased incidence of death-censored loss of both the renal and the pancreatic allograft. Furthermore, a high-MBL status was associated with markedly in-
creased mortality, and we demonstrate that this high-MBL status is genetically determined.

These findings corroborate our recent report demonstrating an association of MBL levels >400 ng/ml with poorer graft survival after deceased-donor kidney transplantation.18 Our earlier study on the role of MBL in kidney transplantation showed a nonsignificant trend toward poorer patient survival in renal allograft recipients with a high MBL level. This difference between the two studies may be explained by the higher risk profile in the patients who had type 1 diabetes and received SPKT compared with the general population of kidney allograft recipients. In addition, the harmful effect of MBL in cardiovascular mortality may be enhanced in the patients with diabetes.

Reports on the cardiovascular effects of MBL deficiency in the general population have been inconclusive. The predictive value of MBL levels for myocardial infarction was studied in the population-based Reykjavik study.20 In this population, MBL levels >1000 ng/ml were associated with a lower odds ratio for myocardial infarction. It is interesting that no data on mortality were reported. In the Strong Heart Study cohort, Native Americans with coronary heart disease had an increased frequency of variant MBL genotypes when compared with a matched cohort without coronary heart disease.21 Contrary to these findings but in agreement with our data in this study, a recent study in 964 seemingly healthy men did show an association of elevated MBL levels with coronary heart disease.22

So how can we explain the adverse effect of high MBL levels on graft and patient survival in our study? The finding of superior graft survival after SKPT confirms our earlier report demonstrating superior allograft survival in recipients with low MBL levels after deceased-donor renal transplantation.18 As in our SPKT cohort, the incidence of acute rejection was similar in the high- and low-MBL groups, but graft loss as a result of rejection occurred much more frequently in recipients with high MBL levels. We hypothesize that MBL contributes to tissue damage in various inflammatory settings, including graft rejection. Models of ischemia/reperfusion damage in heart, intestine, and kidney have shown that MBL A and C–d-deficient mice are protected from ischemia/reperfusion injury as compared with wild-type mice.13,23,24 In line with these findings, MBL deposition was detected in human kidneys with ischemia/reperfusion damage,25 indicating that wild-type MBL may contribute to local complement activation and enhanced inflammation in tissue damage. Next to the interaction of MBL with apoptotic and necrotic cells,26 MBL-mediated damage may also be related to its antibody-binding capacities.27–29 A recent study failed to show an association between MBL levels and patient or graft survival after kidney transplantation.30 In comparison with our studies, it has to be noted that the analysis was performed using the median MBL level or the third quartile as cutoff values, which may not be ideal for detecting MBL-mediated effects.

In addition to the effect of MBL on graft survival, we observed a strong association of high MBL levels with inferior patient survival that was independent of graft survival. Earlier studies pointed toward a detrimental role of MBL in patients with diabetes. High levels of MBL have been associated with an increased frequency of cardiovascular disease and proteinuria in patients with type 1 diabetes.15,16 Similarly, high MBL levels have been related to increased mortality in patients with type 2 diabetes.17 It may well be that MBL exerts a specific harmful

<table>
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<tr>
<th>Risk Factor</th>
<th>Univariate</th>
<th>Multivariateb</th>
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<tbody>
<tr>
<td></td>
<td>HR 95% CI</td>
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<tr>
<td>MBL &gt;400 ng/ml</td>
<td>6.28 1.89 to 20.87</td>
<td>4.44 1.30 to 15.10</td>
</tr>
<tr>
<td>Log MBL ng/ml</td>
<td>2.75 1.24 to 6.11</td>
<td>2.56 1.04 to 6.30</td>
</tr>
<tr>
<td>MBL genotype A/A</td>
<td>3.60 1.22 to 10.06</td>
<td></td>
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<tr>
<td>Significant coronary stenosis</td>
<td>2.00 0.92 to 4.30</td>
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</tr>
<tr>
<td>Male recipient</td>
<td>0.61 0.29 to 1.26</td>
<td></td>
</tr>
<tr>
<td>Recipient age &gt; 40 yr</td>
<td>1.52 0.73 to 3.19</td>
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</tr>
<tr>
<td>Smoking</td>
<td>0.96 0.49 to 2.01</td>
<td></td>
</tr>
<tr>
<td>Baseline cholesterol &gt; 5 mmol/L</td>
<td>0.99 0.47 to 2.09</td>
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</tr>
<tr>
<td>Years diabetes</td>
<td>0.99 0.93 to 1.06</td>
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</tr>
<tr>
<td>CRP</td>
<td>1.03 0.98 to 1.07</td>
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<tr>
<td>MMF versus Azathioprine</td>
<td>0.53 0.22 to 1.31</td>
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<td>Preemptive transplantation</td>
<td>1.25 0.58 to 2.73</td>
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*aCI, confidence interval; HR, hazard ratio; MMF, mycophenolate mofetil.
*bFor the multivariate analysis MBL was adjusted for recipient sex, age and CRP.

Table 2. Risk factors for patient deatha

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*aCI, confidence interval; HR, hazard ratio; MMF, mycophenolate mofetil.
*bFor the multivariate analysis MBL was adjusted for recipient sex, age and CRP.

Table 3. Reason for death according to MBL levels

<table>
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<th>Reason</th>
<th>Recipient MBL Level</th>
<th>P</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>MBL≤400 ng/ml (n = 34)</td>
<td>MBL&gt;400 ng/ml (n = 65)</td>
</tr>
<tr>
<td></td>
<td>n</td>
<td>%</td>
</tr>
<tr>
<td>All causes</td>
<td>3</td>
<td>8.8</td>
</tr>
<tr>
<td>Cardiovascular</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td>Malignancy</td>
<td>1</td>
<td>2.9</td>
</tr>
<tr>
<td>Infection</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td>Other</td>
<td>1</td>
<td>2.9</td>
</tr>
<tr>
<td>Undetermined</td>
<td>1</td>
<td>2.9</td>
</tr>
</tbody>
</table>
effect in the diabetic milieu and the increased mortality in high-MBL patients may be related to microvascular damage obtained before the pancreas transplantation. In addition, the unfavorable effect of MBL observed in the context of ischemia/reperfusion damage may contribute to tissue damage and mortality after cardiovascular events.

Because intraindividual MBL levels are highly stable over time, we are convinced that serum MBL levels measured before transplantation adequately represent the exposition to MBL. Moreover, our MBL assay strongly correlates with both the functional activity of the lectin pathway and the presence of SNP of the MBL gene. In fact, measurement of MBL levels in serum may be a more powerful and convenient method of detecting MBL-mediated effects than genotyping, because not all intraindividual variations in MBL levels are explained by the known polymorphisms of exon 1 and other parts of the MBL gene.

Recently, low MBL levels were related to an increased incidence of clinically important infections after liver transplantation. However, no association between MBL deficiency and infection-related mortality was detected in our cohort. Low infection-related mortality after SPKT has been reported before. Thus, although we cannot exclude that MBL deficiency is associated with an increased incidence of infections after SPKT, this did not contribute to graft survival or patient mortality in our cohort.

MBL levels are a powerful predictor of graft and patient survival after SPKT. If these findings can be confirmed in other study populations, then determination of MBL levels and/or MBL genotyping may aid risk stratification before SPKT. Whether these findings eventually lead to new therapeutic approaches will depend on the elucidation of the underlying pathophysiologic mechanisms.

**CONCISE METHODS**

**Study Population**

Between January 1990 and December 2000, 114 SPKT were performed in the Leiden University Medical Center. All patients had type 1 diabetes. Pretransplantation serum was available from 99 and DNA from 97 of these consecutive recipients. Both pretransplantation serum and DNA were available from 87 of these patients. Pretransplantation sera were routinely obtained at the time of admission for transplantation and stored in aliquots at −80°C. All measurements of MBL were performed in sera that had been frozen and thawed only once. All included patients were regularly followed at our center. None of the 99 patients was lost to follow up. The study was performed according to the guidelines of the ethics committee of the Leiden University Medical Center, and patient anonymity was maintained.

The following clinical data were collected using the Leiden Transplant Database: Donor variables, including gender and age at time point of death; recipient variables (age at time of transplantation, gender, panel-reactive antibodies, cytomegalovirus status, duration of diabetes and dialysis treatment, smoking status, and cholesterol levels); transplantation-related factors (HLA-A, -B, and -DR mismatches; cold ischemia time); and posttransplantation features, including immnosuppressive regimen, occurrence of delayed graft function, acute rejection history, rejection treatment, status of both the kidney and the pancreas allografts, cause of allograft loss, vital status, and cause of death. Rejection was defined as either biopsy-proven rejection or clinical rejection of the kidney with a favorable response to antirejection treatment. Because pancreas rejection is difficult to diagnose and isolated rejection of the pancreas is a rare event, this was not analyzed separately in this study. After transplantation, patients were followed until death or until January 1, 2006. Until May 1995, standard maintenance immunosuppression consisted of prednisone, cyclosporine, and azathioprine. All recipients who received a transplant after May 1995 received prednisolone, cyclosporine, and mycophenolate mofetil. Eighteen patients received induction treatment with OKT-3 between 1991 and 1994. From 1999 onward, induction treatment was reinitiated and consisted of either polyclonal antithymocyte globulin (Fresenius, Bad Homburg, Germany) or daclizumab (n = 19). Acute rejection episodes were treated according to a standard protocol consisting of methylprednisolone 1 g intravenously for 3 consecutive days; a 10-d course of antithymocyte globulin at a dosage of 5 mg/kg guided by absolute lymphocyte counts; or again methylprednisolone for the first, second (or steroid-resistant), or third rejection episode, respectively.

**ELISA**

Serum MBL levels were assessed by sandwich ELISA as described previously. In brief, 96-well ELISA plates (Greiner, Frickenhausen, Germany) were coated with the mAb 3E7 (mouse IgG1 anti-MBL at 2.5 μg/ml; provided by Dr. T. Fujita, Fuhushima, Japan). Serum samples were diluted 1:50 and 1:500 and incubated in the coated wells. MBL was detected with Dig-conjugated 3E7. Detection of binding of Dig-conjugated antibodies was performed using horseradish peroxidase–conjugated sheep anti-Dig antibodies (Fab fragments; Roche, Mannheim, Germany). Enzyme activity was detected using 2,2'-azinobis(3-ethylbenzthiazoline-6-sulfonic acid) (Sigma Chemical Co., St. Louis, MO). The optical density was measured at 415 nm using a microplate biokinetics reader (EL312e; Biotek Instruments, Winooski, VT). A calibration line was produced using human serum from a healthy donor with a known concentration of MBL. Earlier studies indicated that this assay primarily detects wild-type MBL in serum and plasma and that there is a direct association with the MBL genotype and with MBL function.

**Genotyping**

DNA from 97 SPKT recipients was isolated routinely from blood. MBL SNP at codons 52, 54, and 57 of the mbl2 gene were typed by high-resolution DNA melting analysis. The detailed method will be published separately (A.R. et al., manuscript in preparation). The MBL genotype of only wild-type allele carriers is designated as A/A, and the presence of one or two variant alleles (B, C, or D) is designated as A/O or O/O. In the survival analysis, carriers of A/O and O/O MBL genotype were considered as one group.
Statistical Analyses
Categorical characteristics were compared using cross-tables with calculation of the exact P values. Interval variables were analyzed using the independent-samples t test when assumptions for parametric testing were met. Otherwise, the Mann-Whitney U test was used. Patient and graft survival was estimated using the Kaplan-Meier product-limit method, and the curves were compared with the log-rank test. For both pancreas and renal allograft survival, the analysis was censored for patient death. Organs that were lost as a result of technical failure or thrombosis within 1 wk after transplantation were excluded from survival analysis.

Cox proportional hazards regression was used to identify possible confounders influencing baseline MBL levels. In the multivariate model, MBL was adjusted for recipient age, gender, and baseline CRP level. MBL was tested both as a dichotomous (MBL < or >400 ng/ml) and as a continuous factor (after log transformation). P < 0.05 was considered to be significant. Data analysis was performed with SPSS Statistical Software Package (version 11.0; SPSS, Chicago, IL).

ACKNOWLEDGMENTS
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DISCLOSURES
None.

REFERENCES
23. Hart ML, Ceonzo KA, Shaffer LA, Takahashi K, Rother RP, Reenstra


