

# Prevalence of Cryoglobulinemia and Autoimmune Markers in Liver Transplant Patients

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## Abstract

**Objectives:** To examine the prevalence of cryoglobulinemia and autoimmune markers in stable liver transplant recipients and to determine risk factors and clinical impact.

**Materials and Methods:** Ninety-two liver transplant recipients were tested for cryoglobulinemia, hepatitis B and C, complement C3, complement C4, CH50, antinuclear antibodies, anticytoplasmic neutrophil antibodies, anticardiolipid antibodies, rheumatoid factors, and lymphocyte subpopulations. Liver, renal, and hematology tests were done. Immunosuppressive regimens were based on calcineurin inhibitors in 94.6% of the patients.

**Results:** Cryoglobulinemia was present in 18 patients (19.5%) with characteristics of type II in 27.7%, type III in 61.3%, and indeterminate in 11%. Cryoglobulinemia was present in 55.5% of patients with positive hepatitis C virus serology compared with 35.86% of patients with negative hepatitis C virus serology ( $P = .06$ ). Among those with hepatitis C virus markers, cryoglobulinemia was present in 30%. Anticytoplasmic neutrophil antibodies were positive in 23% of the patients with cryoglobulinemia, but in only 5.4% of the patients without cryoglobulinemia ( $P = .006$ ). Albuminemia was significantly lower in patients with cryoglobulinemia ( $38 \pm 4.2$  g/L) than it was in patients

without cryoglobulinemia ( $40.2 \pm 3.4$ ;  $P = .05$ ). Cryoglobulinemia was symptomatic in 4 patients (22.2% of all patients). Independent factors associated with cryoglobulinemia were presence of anticytoplasmic neutrophil antibodies, more than 4 HLA incompatibilities, alanine aminotransferase level of  $0.68 \mu\text{kat/L}$  or more, and an albuminemia level greater than 38 g/L.

**Conclusions:** Cryoglobulinemia is frequent after liver transplant and is symptomatic in approximately 20% of all patients.

**Key words:** Alanine aminotransferase, Anticytoplasmic neutrophil, Cyclosporine, Hepatitis C virus, Tacrolimus

Cryoglobulins are single or mixed immunoglobulins that reversibly precipitate at low temperatures (1) and might be related to hepatitis C virus (HCV), autoimmune diseases, lymphoid hemopathies, infections, and renal and liver diseases (2). The pathophysiology of cryoglobulinemia involves B-cell clonal expansion and can be considered an autoimmune disorder. Cryoglobulinemia has been suspected in cases of systemic vasculitis associated with renal membranous and membranoproliferative glomerulonephritis, as well as cutaneous (eg, purpura, cold urticaria, leg ulcers, or Raynaud's phenomenon), neurologic (eg, peripheral neuropathy), and rheumatologic (eg, arthralgia) manifestations. In patients who have not had an organ transplant, cryoglobulinemia is a common feature in patients with HCV, with incidences of 40% to 100% (3-5). In kidney transplant patients, the incidence of cryoglobulinemia is estimated at 2% to 74.4% (6-9), with an incidence of 37.8% and 27% in patients with and without HCV (9). Two studies of liver transplant recipients with HCV published 5 and 7 years ago found a prevalence of cryoglobulinemia to be 19% and 30%, respectively (10, 11).

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Serum cryoglobulin values usually do not correlate with clinical severity and prognosis of the disease: low cryocrit values can be associated with a severe active cryoglobulinemia syndrome, and patients with high serum cryoglobulin concentrations may be completely asymptomatic (2). However, in the individual patient, cryoglobulin level may serve as a marker for the disease.

Rituximab therapy has been successfully used in cryoglobulin-associated glomerulonephritis, both on native (12, 13) and transplant kidneys (14, 15). However, in patients in whom cryoglobulinemia is found after kidney or liver transplant, it is not known if treatment is necessary. Thus, understanding the risk factors for cryoglobulinemia, as well as its clinical and biological consequences in liver transplant patients, is of clinical importance.

We examined the prevalence of cryoglobulinemia and autoimmune markers and their clinical implications in 92 liver transplant patients who consecutively attended our outpatient clinic from May 10 through May 26, 2007.

## Materials and Methods

### *Patients*

We tested for cryoglobulinemia and autoimmune markers in all liver transplant patients who attended our outpatient clinic between May 10 and May 26, 2007. Criteria for inclusion were that patients had to have received a transplant more than 12 months earlier and were not participating in another clinical trial at the time of this study. Ninety-two liver transplant patients (25 women, 67 men, aged, 23-76 years) were included. Five patients (5.4%) were the recipient of a second transplant. With respect to end-stage liver disease on the native liver, patients were divided into 3 categories: (1) those with HCV-related end-stage liver disease (n=34), (2) those with autoimmune-related end-stage liver disease (n=8), and (3) those who had received a transplant for other reasons (n=50). These 92 patients were representative of the entire cohort of liver transplant patients still alive at the time of the study.

Of the 34 HCV-positive liver transplant patients, 18 (53%) were HCV RNA positive at the time of the study. The median time between liver transplant and autoimmune marker/cryoglobulinemia tests was 62.4 months (range, 8-238 months). On the test day, the patient's immunosuppression regimen consisted of

either calcineurin-inhibitor immunosuppression (85 patients [94.6%], ie, tacrolimus in 62 [67%] of 92 patients and cyclosporine in 23 [25%] of 92 patients) or calcineurin-inhibitor-free immunosuppression (sirolimus-based, 6 patients; mycophenolate-mofetil-based, 1). In addition to previous immunosuppressive agents, 34 patients (37%) were receiving mycophenolate mofetil, and 37 patients (40.2%) were receiving steroids.

We sent a questionnaire to all patients in whom cryoglobulin was found. This questionnaire sought information concerning symptoms that might be related to the presence of cryoglobulin, for example, cutaneous lesions, Raynaud's syndrome, arthralgias, paresthesias, and myalgias. When the questionnaire was complete, the patients were contacted by one of the researchers (CG) to validate any reported symptoms.

### *Laboratory tests*

The following biological variables were assessed: liver enzymes (including alanine aminotransferase and aspartate aminotransferase, gamma glutamyl transpeptidase, alkaline phosphatase, and total bilirubin); kidney variables (ie, serum creatinine, calculated creatinine clearance [Cockcroft-Gault formula], albuminemia, and microalbuminuria); hematologic tests (hemoglobin level, platelet and leucocyte counts, and lymphocyte subpopulations); viral tests (hepatitis C and B virus, ie, serology and nucleic acid testing, where appropriate, and cytomegalovirus [CMV] and Epstein-Barr virus DNAemia); and immunologic tests (serum complement, ie, C3, C4, and CH50 levels; rheumatoid factors; immunoelectrophoresis; antinuclear antibodies [ANA assessed on Hep2 cells; positive if the titer was > 1/80], and anticytoplasmic neutrophil antibodies [ANCA; positive if the titer was > 1/100], anticardiolipid antibodies, and cryoglobulinemia). Owing to a lack of stored frozen plasma, we were unable to retrospectively assess the presence or absence of autoantibodies before liver transplant. Cryoglobulinemia was not looked for before transplant.

*Cryoglobulinemia assessment:* Cryoglobulinemia testing was done as follows: 7 mL blood was collected in a tube that had been prewarmed to 37°C. The blood was then kept at 37°C before being transferred to the laboratory for centrifugation. Samples were allowed to clot at 37°C for 4 hours before separation. Serum was

separated from the clot by centrifuging while still warm for 10 minutes at 3500 rpm. After separation, 1 drop of 0.1 g sodium azide per liter was added to the sample to prevent bacterial overgrowth. The serum was then tested for cryoprecipitation by inspection after 7 days at 4°C and compared with a sample from a separate aliquot kept at 37°C (16). After rigorously washing the cryoglobulin, quantification of cryoglobulin was done by measuring optical density at 280 nm. The detection threshold was 10 mg/L. Typing of the isolated cryoglobulin was done by immunoblotting. Cryoglobulins were fractionated on an agarose gel (Paragon Serum Protein Electrophoresis kit, Beckman Coulter, Inc., Fullerton, CA, USA), and probed with antisera to immunoglobulin for the heavy- and light-chain determinants (Dako A/S, Glostrup, Denmark) (17).

### Statistical analyses

Results are presented as means  $\pm$  standard deviation, or as median (ranges) where appropriate. Comparisons of qualitative variables were done with the chi-square test; comparisons of quantitative variables were done with the Wilcoxon signed rank or *t* test, when appropriate. A value for *P* less than .05 was considered statistically significant.

Multivariate analyses were done by logistic regression to assess factors associated with the presence of cryoglobulinemia. For that analysis, quantitative variables were transformed into qualitative variables. For each variable, the median value was chosen as the cutoff value.

### Results

Cryoglobulinemia was positive in 18 of 92 patients (19.5%), with characteristics of type II in 27.7%, of type III in 61.3%, and indeterminate in 11% of patients. The median cryoglobulin level was 133.2 mg/L (range, 10-379 mg/L); that is, 353 mg/L (range, 10-840 mg/L) for type II and 69.3 mg/L (range, 10-179 mg/L) for type III. Hepatitis C virus serology was positive in 55.5% of patients with cryoglobulinemia compared with only 35.8% of those without cryoglobulinemia (*P* = .06). Among those with HCV markers, cryoglobulinemia was present in 30%. In patients with cryoglobulinemia, calcineurin inhibitors were tacrolimus in 10 of 18 (55.6%) and cyclosporine in 6 of 18 patients (33.3%) compared with 52 of 74 (70.3%) and 17 of 74 patients (23%) without cryoglobulinemia, respectively (*P* = NS).

Sirolimus was used in 1 patient (5.6%) with cryoglobulinemia compared with 5 patients (6.8%) without cryoglobulinemia. Mycophenolate acid was used in 33.3% of patients with cryoglobulinemia compared with 37.8% of patients without cryoglobulinemia, and steroids were used in 44.4% of patients with cryoglobulinemia compared with 39.2% of patients without cryoglobulinemia (*P* = NS).

Immunoelectrophoresis showed oligoclonal hypergammaglobulinemia in 3 of 18 patients (16.7%), monoclonal hypergammaglobulinemia in 1 of 18 patients (5.6%), and was normal in 14 patients with cryoglobulinemia (77.8%) compared with 12 of 74 (16.2%) with oligoclonal hypergammaglobulinemia, 5 of 74 (6.8%) with monoclonal hypergammaglobulinemia, and 57 of 74 (77%) with normal results on immunoelectrophoresis in patients without cryoglobulinemia; these results were not statistically significant. Rheumatoid (antihuman IgG) factors was positive in 11 of 18 (61.1%) patients with cryoglobulinemia and in 37 of 74 patients (50%) without cryoglobulinemia, with a similar titer found in patients with cryoglobulinemia (34 IU/mL [0-261 IU/mL] vs 20 IU/mL [0-346 IU/mL]) (*P* = NS). Anticytoplasmic neutrophil antibodies were positive in 23% of patients with cryoglobulinemia, but in only 5.4% of patients without cryoglobulinemia (*P* = .006). Antinuclear antibodies were positive in 50% of patients with cryoglobulinemia compared with 43.2% of patients without cryoglobulinemia (*P* = NS). Anticardiolipid antibodies were positive in only 1 patient from each group. The calculated creatinine clearance rate was similar in patients with (61.5  $\pm$  17 mL/min) and without (61.8  $\pm$  20.5 mL/min) cryoglobulinemia (*P* = NS). Microalbuminuria was similar in those with cryoglobulinemia (13 mg/L [2-1090 mg/L]) and without cryoglobulinemia (11 mg/L [2-633 mg/L]). Albuminemia was significantly lower in patients with cryoglobulinemia (38  $\pm$  4.2 g/L) than it was in patients without cryoglobulinemia (40.2  $\pm$  3.4 g/L; *P* = .05).

There was no significant difference between patients with and without cryoglobulinemia in terms of age, sex, human leukocyte antigen mismatch, daily steroid dosage, platelet and leukocyte counts, hemoglobin level, pretransplant panel-reactive antibodies, serum complement levels (CH50, C3, C4), lymphocyte subpopulations (ie, CD2, CD3, CD4, CD8, CD19 subsets), and hepatitis B virus serology (Tables 1 and 2). With respect to liver enzymes, the

only statistically significant difference was regarding aspartate aminotransferase, which was  $0.42 \mu\text{kat/L}$  (range,  $0.42\text{--}13.55 \mu\text{kat/L}$ ) in those without cryoglobulinemia compared with  $0.69 \mu\text{kat/L}$  (range,  $0.3\text{--}2.99 \mu\text{kat/L}$ ) in patients with cryoglobulinemia ( $P = .01$ ). Finally, CMV DNAemia was positive in only 3 patients (2 patients with cryoglobulinemia and 1 without cryoglobulinemia), whereas Epstein-Barr virus DNAemia was positive in 25% of those without cryoglobulinemia and in 28.6% of patients with cryoglobulinemia ( $P = \text{NS}$ ). The Epstein-Barr virus

quantitative viremia levels were similar in both groups (Table 3).

**Table 1.** Demographic data according to the presence or absence of cryoglobulinemia.

|                                        | Cryo(+) patients (n=18) | Cryo(-) patients (n=74) | P value |
|----------------------------------------|-------------------------|-------------------------|---------|
| Age (years)                            | 57 $\pm$ 13             | 57 $\pm$ 10             | NS      |
| Sex (M/F)                              | 15/3                    | 51/23                   | NS      |
| BMI (kg/m <sup>2</sup> )               | 25.4 $\pm$ 4.4          | 25 $\pm$ 4.4            | NS      |
| Time since OLT (months)                | 60 $\pm$ 50             | 63 $\pm$ 51             | NS      |
| Serum creatinine ( $\mu\text{mol/L}$ ) | 117 $\pm$ 28.7          | 121.5 $\pm$ 39.7        | NS      |
| Cause of end-stage liver disease       |                         |                         |         |
| HCV, n (%)                             | 10 (55.5)               | 24 (32.4)               | .06     |
| Autoimmunity, n (%)                    | 2 (11.1)                | 7 (9.4)                 | NS      |
| Other, n (%)                           | 6 (33.4)                | 43 (58.2)               | NS      |
| Second OLT, n (%)                      | 0 (0)                   | 5 (6.7)                 | NS      |
| One AR or more, n (%)                  | 7 (38.8)                | 30 (40.5)               | NS      |
| HLA ABDR incompatibilities             | 4 (3-6)                 | 5 (2-6)                 | NS      |
| PRA > 0, n                             | 1                       | 6                       | NS      |
| Anti-class I alloAb, n                 | 1                       | 4                       | NS      |
| Anti-class II alloAb, n                | 1                       | 6                       | NS      |

**Abbreviations:** alloAb, alloantibodies; AR, acute rejection; BMI, body mass index; Cryo, cryoglobulinemia; ESRD, end-stage liver disease; F, female; HCV, hepatitis C virus; HLA, human leukocyte antigens; M, male; NS, not significant; OLT, orthotopic liver transplant; PRA, panel-reactive alloantibodies.

**Table 2.** Immunologic, biological, and hematologic variables according to presence or absence of cryoglobulinemia.

|                                                   | Cryo(-) patients (n=74) | Cryo(+) patients (n=18) | P value |
|---------------------------------------------------|-------------------------|-------------------------|---------|
| RF, n (%)                                         | 37 (50)                 | 11 (61.1)               | NS      |
| RF titer (IU/mL)                                  | 20 (0-346)              | 34 (0-261)              | NS      |
| Complement fractions:                             |                         |                         |         |
| CH50                                              | 529 $\pm$ 95            | 511 $\pm$ 89            | NS      |
| C3 (g/L) (n, 0.8-1.57)                            | 1 $\pm$ 0.25            | 1 $\pm$ 0.24            | NS      |
| C4 (g/L) (n, 0.19-0.6)                            | 0.23 $\pm$ 0.07         | 0.22 $\pm$ 0.05         | NS      |
| ANCA (+), n (%)                                   | 4 (5.4)                 | 3* (23)                 | .006    |
| ANA (+), n (%)                                    | 32 (43.2)               | 9 (50)                  | NS      |
| Anticardiolipin Ab (+), n (%)                     | 1                       | 1                       | NS      |
| AST ( $\mu\text{kat/L}$ )                         | 0.42 (0.18-13.55)       | 0.69 (0.3-2.99)         | .01     |
| ALT ( $\mu\text{kat/L}$ )                         | 0.47 (0.13-6.58)        | 0.86 (0.24-4.18)        | NS      |
| Microalbuminuria (mg/L)                           | 11 (2-633)              | 13 (2-1090)             | NS      |
| Creatinine clearance (mL/min)                     | 61.8 $\pm$ 20.5         | 61.6 $\pm$ 16.9         | NS      |
| Hemoglobin (g/dL)                                 | 13.4 $\pm$ 1.8          | 13.7 $\pm$ 1.7          | NS      |
| Platelets ( $\times 10^3$ cells/mm <sup>3</sup> ) | 170 (60-177)            | 148 (67-321)            | NS      |
| WBCC (cells/mm <sup>3</sup> )                     | 5970 (1330-15280)       | 5850 (3140-8250)        | NS      |

**Abbreviations:** ANA, antinuclear antibodies, ANCA, anticytoplasmic nuclear antibodies, AST, aspartate aminotransferase; ALT, alanine aminotransferase; C3, complement factor C3; C4, complement factor C4; Cryo, cryoglobulinemia; RF, rheumatoid factor; WBCC, white blood-cell count.

\*this was tested in only 13 patients.

**Table 3.** Virologic variables according to the presence or absence of cryoglobulinemia.

|                             | Cryo(-) patients (n=74) | Cryo(+) patients (n=18) | P value |
|-----------------------------|-------------------------|-------------------------|---------|
| HCV(+) antibodies: n (%)    | 24 (32.4)               | 10 (55.6)               | .06     |
| HCV RNA(+), n (%)           | 13 (54.2)               | 5 (50)                  | NS      |
| HCV RNA (log copies/mL)     | 5.8 $\pm$ 0.79          | 6.64 $\pm$ 0.69         | .07     |
| HBs antigen-positive        | 3                       | 1                       | NS      |
| EBV(+) viremia (%)          | 25                      | 28.6                    | NS      |
| EBV viremia (log copies/mL) | 3.39 $\pm$ 0.43         | 3.44 $\pm$ 0.78         | NS      |
| CMV(+) viremia (%)          | 3.3                     | 6.7                     | NS      |

**Abbreviations:** Cryo, cryoglobulinemia; CMV, cytomegalovirus; EBV, Epstein-Barr virus; HCV, hepatitis C virus; HBs, hepatitis B virus surface antigen; NS, not significant.

We did a multivariate analysis to determine the factors that were independently associated with developing cryoglobulinemia. The following factors were associated with cryoglobulinemia: presence of ANCA (OR 17.3; 95% CI: 1.85-162) ( $P = .01$ ); human leukocyte antigen incompatibilities greater than 4 (OR 0.21; 95% CI: 0.04-0.97) ( $P = .046$ ), alanine aminotransferase level greater or equal to  $0.68 \mu\text{kat/L}$  (OR 9.12; 95% CI: 1.91-43.5) ( $P = .005$ ), and albuminemia > 38 g/L (OR 0.07; 95% CI: 0.01-0.42) ( $P = .003$ ) (Table 4).

**Table 4.** Multivariate analysis of predictive factors for the presence of cryoglobulinemia after liver transplant.

| Variables                                   | Point estimate: (95% confidence limits) | P value |
|---------------------------------------------|-----------------------------------------|---------|
| Neutrophil anticytoplasmic antibodies (yes) | 17.3 (1.85-162)                         | .01     |
| Number of HLA mismatches (> 4)              | 0.21 (0.04-0.97)                        | .046    |
| ALT greater than $0.68 \mu\text{kat/L}$     | 9.1 (1.91-43.5)                         | .005    |
| Albuminemia > 38 g/L (yes)                  | 0.07 (0.01-0.42)                        | .003    |

**Abbreviations:** ALT, alanine aminotransferase; HLA, human leukocyte antigen

Among the 18 patients with cryoglobulinemia, only 4 presented with clinical signs (according to Ferri criteria) (18) of which 3 had type III cryoglobulinemia, and 1 had type II cryoglobulinemia (Table 5). Thus, 20% of the patients with type II and 27.3% of the patients with type III cryoglobulinemia had cryoglobulinemia-related symptoms. Of these 4 patients, 3 (Nos. 1, 2, and 3) had an ongoing HCV infection as demonstrated by positive RNA. Component factors C3 and C4 were normal in all 4 patients, and all had high rheumatoid-factor titers. In addition, 2 patients (Nos. 3 and 4) had high ANA titers, although anti-DNA ds, -SSA, -SSB, -Sm, -RNP, -Scl70, -Jo1, -centromere, and ribosome antibodies were all negative. One patient (No. 3) had nephrotic-range proteinuria (5 g/d), whereas albuminuria was mild in

**Table 5.** Cryoglobulinemic liver transplant patients for whom cryoglobulinemia was symptomatic.

|                                                   | Case 1                                                                | Case 2             | Case 3                                    | Case 4                    |
|---------------------------------------------------|-----------------------------------------------------------------------|--------------------|-------------------------------------------|---------------------------|
| Cryoglobulinemia level (mg/L)                     | 840                                                                   | 26                 | 133                                       | 10                        |
| Type (II or III)                                  | II                                                                    | III                | III                                       | III                       |
| Cause of liver disease                            | HCV (+)/RNA (+)                                                       | HCV (+)/RNA (+)    | HCV (+)/RNA (+)                           | Primary biliary cirrhosis |
| HCV RNA (log copies/mL)                           | 6.4                                                                   | 6.2                | 6.15                                      | NA                        |
| Anti-HCV therapy within 6 months of the Cryo test | No                                                                    | No                 | No                                        | NA                        |
| C3 (g/dL) (0.8-1.57)                              | 1.05                                                                  | 0.79               | 0.77                                      | 0.89                      |
| ANA titer                                         | 0                                                                     | 0                  | 1/2500                                    | 1/1280                    |
| Rheumatoid factor (IU/mL)                         | 125                                                                   | 58                 | 261                                       | 6060                      |
| Serum creatinine ( $\mu$ mol/L)                   | 151                                                                   | 123                | 116                                       | 94                        |
| Microalbuminuria (mg/L)                           | 890                                                                   | NA                 | 5000                                      | 20                        |
| Clinical signs                                    | Asthenia, palpable purpura, peripheral neuropathy, Raynaud's syndrome | Raynaud's syndrome | Membrano-proliferative glomerulonephritis | Arthralgias               |

**Abbreviations:** ANA, antinuclear antibody; Cryo, cryoglobulinemia; HCV, hepatitis C virus; C3, complement factor C3; NA, not applicable.

the others, ranging from 2 to 89 mg/L. The patient with nephrotic syndrome (proteinuria, 5 g/d; serum creatinine, 116  $\mu$ mol/L) was given rituximab therapy (375 mg/m<sup>2</sup> weekly for 2 consecutive weeks). This resulted in a dramatic decrease in the cryoglobulin level from 133 mg/L to less than 10 mg/L, and a sustained decrease in proteinuria from 5 g/d to 1.5 g/d. Among patients with cryoglobulinemia, we were unable to predict the type or level of deleterious and nondeleterious cryoglobulinemia as far as cryoglobulinemia-related symptoms were concerned.

## Discussion

We examined the prevalence of cryoglobulinemia in liver transplant recipients in stable condition and tried to determine the risk factors for cryoglobulinemia. We found a high prevalence (19.5%) of cryoglobulinemia: most patients had type III cryoglobulinemia. However, this figure is much lower than that reported for kidney transplant recipients (approximately 40%) (6-9). The number of cases we found in our orthotopic liver transplant patients is similar to that reported by Abrahamian and associates (10) (19%) in a small cohort of 52 liver transplant patients, although these authors found cryoglobulinemia only in HCV-positive patients, whereas in our study, 8 of 18 patients with cryoglobulinemia had no markers for HCV (negative serology as well as negative HCV RNA).

The second study to address this topic in liver transplant patients looked for cryoglobulinemia in a group of 30 HCV-positive liver transplant patients and found a prevalence of 30% (11). This prevalence rate is similar to that observed in our HCV-positive patients (30%). Because we included many more patients

(n=92) than the 2 previous studies did, we could look for the independent predictive factors for developing cryoglobulinemia.

Among the predictive factors for cryoglobulinemia, we found that having ANCAs increased risk more than 17 times. This was independent of the cause of end-stage liver disease; that is, it was not linked to autoimmune hepatitis (19) or primary sclerosing cholangitis (20), both of which are known to be associated with ANCAs. This could reflect the fact that liver transplant patients with cryoglobulinemia might have some degree of B-lymphocyte function dysregulation, which results in production of auto-antibodies and cryoglobulins independently of HCV infection or hepatic autoimmune disease. Other predictors for cryoglobulinemia were alanine aminotransferase levels higher than 0.68  $\mu$ kat/L, a well-matched human leukocyte antigen allograft, and decreased albuminemia levels. However, we have no clear explanations for these predictors.

In patients who did not undergo an organ transplant, type III cryoglobulinemia may be observed in many situations, such as in inflammatory or infectious diseases (21). In these situations, type III cryoglobulinemia does not induce systemic vasculitis. Conversely, type II cryoglobulinemia might be associated with life-threatening symptoms (22). In our study, most patients with cryoglobulinemia had type III cryoglobulinemia. As opposed to those who have not had an organ transplant, 27.3% of the liver transplant patients with type III cryoglobulinemia had cryoglobulinemia-associated symptoms including membranoproliferative glomerulonephritis. The latter is typically associated with type II cryoglobulinemia in persons with HCV infection

who have not had an organ transplant (23, 24).

What are the driving factors for developing cryoglobulinemia? In 1986, Fiorini and colleagues (25) addressed the frequency of antibodies produced in response to ubiquitous viruses such as Epstein-Barr virus, CMV, and HBV. These authors found that antibodies to CMV and HBV were no more frequent in patients with cryoglobulinemia than they were in control subjects. Conversely, IgM antibodies to the viral capsid-antigen of Epstein-Barr virus were found in 11 of 13 patients with cryoglobulinemia. The authors wondered whether there was a possible correlation between cryoglobulinemia and Epstein-Barr virus infection. However, in our study we assessed both CMV and Epstein-Barr virus DNAemias. Even if some patients tested positive for 1 of these viruses, positive DNAemias did not correlate with presence of cryoglobulinemia. Therefore, we did not find a correlation between Epstein-Barr virus infection and cryoglobulinemia.

We were unable to predict the type or the level of deleterious and nondeleterious cryoglobulinemia using the standard immunological markers. Therefore, we believe that there is no need to screen liver transplant patients for the presence of cryoglobulinemia. However, when liver transplant patients present with symptoms that are potentially associated with the presence of cryoglobulinemia (eg, cutaneous lesions, Raynaud's syndrome, arthralgias, paresthesias, and myalgias), cryoglobulinemia should be looked for because, if present, one might contemplate modifying the immunosuppressive therapy by implementing rituximab therapy.

Cryoglobulinemia is frequent after liver transplant, but is symptomatic only in approximately 20% of patients. Therefore, it is not mandatory to systematically screen liver transplant patients for cryoglobulinemia.

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