Renal Transplantation Relieves the Symptoms but Does Not Reverse \(\beta_2\)-Microglobulin Amyloidosis

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(J. Am. Soc. Nephrol. 1996; 7:798-804)

**ABSTRACT**

Renal transplantation is considered to be the treatment of choice of dialysis-related \(\beta_2\)-microglobulin amyloidosis (DRA), as it provides near-normal serum levels of \(\beta_2\)-microglobulin and obviates the need for dialysis. However, the long-term outcome of DRA after transplantation has not been fully assessed, and its evolution after transplant failure has not been reported. This study examined 17 patients with histologically confirmed DRA who underwent kidney transplantation and had a dialysis-free follow-up period in excess of 1 yr. Immunosuppressive treatment included low-dose prednisolone, cyclosporine, and/or azathioprine. Symptoms related to DRA were sought at every outpatient visit, and bone x-rays were performed at time of transplantation and annually thereafter. The number and size of the bone cysts were determined. Most of the DRA symptoms, and particularly shoulder stiffness, disappeared within the first wk after transplantation and this persisted throughout the transplant follow-up period (58.5 ± 9 months). However, the number of bone cysts remained remarkably constant even in those patients with still-functioning grafts (12 ± 7.5 and 12.1 ± 7.7, before and at last transplantation follow-up examination, respectively). \(\beta_2\)-microglobulin amyloid was found to be present in one patient operated on for hip fracture 2 yr after receiving a well-functioning transplant. Seven patients experienced graft failure and returned to dialysis after 47 ± 39 months of transplantation. Severe DRA symptoms reappeared strikingly early after resuming hemodialysis, and five out of the seven patients required surgery for carpal tunnel syndrome, three of them within the first yr (mean, 17 ± 12 months). The number of cysts significantly increased from 17 ± 11 to 21 ± 11 during the second dialysis period. These findings provide further evidence suggesting that although the clinical expression of DRA is arrested during transplantation, the anatomical lesions and the pathological processes underlying it are unlikely to be reversed.

Key Words: Dialysis-related amyloidosis, carpal tunnel syndrome, bone cysts, renal transplantation

\(\beta_2\)-Microglobulin amyloidosis is a well-known complication frequently found in dialysis patients. Two independent factors increase the risk of this complication: patient age and duration of dialysis treatment (1,2). The prevalence of \(\beta_2\)-microglobulin amyloidosis has been reported to be more than 50% at 165 months of dialysis treatment and as high as the near totality of patients over 20 yr of dialysis treatment (2). Usually, dialysis-related \(\beta_2\)-microglobulin amyloidosis (DRA) involves bone and joint structures and results in painful and disabling symptoms such as carpal tunnel syndrome (CTS), shoulder stiffness, and bone cysts with fractures (2-4). No effective treatment or prevention is available at the present time for DRA; switching patients to high-flux membranes (in hemofiltration, hemodiafiltration, or hemodialysis) has been proposed (5). However, although these treatments lower \(\beta_2\)-microglobulin serum levels and retard the occurrence or the severity of this complication, there is no convincing evidence that they completely prevent DRA.

When patients with DRA are successfully transplanted, \(\beta_2\)-microglobulin levels fall to near normal. By obviating the need for dialysis, transplantation has been proposed as the treatment of choice for DRA. However, whether amyloid deposits may disappear is still controversial. Although it was initially suggested that amyloid dissolution might occur (6,7), subsequent radiological studies seem to demonstrate the persistence of bone amyloid cysts (8,9), and one case of \(\beta_2\)-microglobulin CTS has been reported 28 months after transplantation (10). Indeed, the long-term outcome of DRA after transplantation has not been fully assessed, and its evolution after transplant failure has not been previously reported.

In the study presented here, we assessed the effect of renal transplantation in 17 patients with histologically proven DRA. After a mean follow-up period of 6 yr, we observed that in the ten transplant recipients with ongoing satisfactory graft function, there is no clinical evidence of progression or anatomical regression of DRA, whereas in those seven patients who returned to hemodialysis, a quick and severe relapse of amyloid symptoms appeared.
PATIENTS AND METHODS

Patients

The inclusion criteria for the study were: (1) histologically proven β2-microglobulin amyloidosis; (2) renal transplantation with satisfactory graft function (serum creatinine concentration lower than 200 μmol/L after the first month after transplantation); and (3) a dialysis-free period in excess of 1 yr. Among the 764 patients who underwent renal transplantation at our institution between January 1980 and December 1992, 17 fulfilled these criteria. The histological diagnosis of DRA had been performed on material removed during surgery, including synovia, tendons, and bone cysts. None suffered primary amyloidosis, multiple myeloma, or other systemic disease known to cause articular symptoms. These patients were transplanted after 92 to 238 months of hemodialysis and their clinical characteristics are depicted in Table 1. In fact, they became candidates for transplantation after our transplant program was strengthened and patient and graft survival significantly improved. All patients were initially dialyzed with cellulose membranes and five of them were switched to high-flux membranes before transplantation. All suffered from severe osteoarticular pain at the time of transplantation: CTS in 15 patients (14 bilateral, 1 unilateral); multiple bone cysts involving primarily wrists, shoulders, or hips in 17 patients; and stiffness of the shoulders in 12. One patient had a history of previous femoral neck fracture, and the femoral head removed during surgery contained a large amyloid-containing cyst.

Immunosuppressive Treatment

Induction immunosuppression included antithymocyte globulins (ALG) (Merieux Institute, Lyon, France), azathioprine (2 mg/kg per day) and low-dose prednisolone (20 mg/day). Before 1985, ALG was stopped at Day 21 and CsA was used in our center as monotherapy; after 1985, maintenance immunosuppression included prednisolone (5 to 10 mg/day at 1 yr) and CsA (dose adjusted to maintain a trough level of 150 ng/mL during the second and third months and to 100 ng/mL thereafter); azathioprine was stopped at 6 months. Acute rejection episodes were treated with IV methylprednisolone: a single steroid-resistant rejection was treated with the monoclonal antibody OKT3 (Ortho Laboratories, Raritan, NJ).

Clinical Follow-Up

Patients were regularly monitored at our outpatient clinic (1 visit/wk for 3 months, 1 visit/month during 9 months, and quarterly thereafter). At each visit, body weight, blood pressure, clinical status, and osteoarticular complaints were monitored. A clinical score ranging from 0 to 4 was defined to semiquantitatively estimate shoulder stiffness (0, no pain; 1, mild pain not requiring treatment; 2, pain requiring intermittent analgesics; 3, pain requiring permanent treatment with nonsteroidal anti-inflammatory agents; 4, pain resulting in insomnia and/or depression despite treatment). Serum creatinine concentration, proteinuria, CsA concentration, and blood cell counts were registered. β2-Microglobulin serum levels were monitored every year. When transplant failure occurred and renal replacement therapy was re instituted, we continued to monitor the patients regularly.

Radiological Follow-Up

Joint x-rays were obtained in all patients at the time of transplantation and annually thereafter. They included anteroposterior and lateral views of the wrists, shoulders, and hips. The x-rays were reviewed independently by two trained observers, and each significant cyst was recorded (>3 mm in diameter in the wrists and >5 mm in diameter in the shoulders and hips).

Amyloid Studies

Conventional histological methods (Congo red and thioflavine T stainings) were performed in all of the osteoarticular material obtained from these patients, either during the hemodialysis or transplantation periods, to assess β2-microglobulin amyloidosis. Biochemical studies included protein characterization and identification with sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), two-dimensional gel electrophoresis, and Western blotting, as we have previously reported (11).

Statistical Methods

Data are given as mean ± SD of the mean. The differences between groups were assessed with the Mann-Whitney's U test and the differences for time points were assessed with Wilcoxon's test for paired data. A P value lower than 0.05 was considered significant.
RESULTS

Postoperative Period

Four patients experienced acute tubular necrosis requiring hemodialysis after transplantation (three to six hemodialysis sessions). At 1 month after transplantation, the mean plasma creatinine concentration was $153 \pm 35 \mu M/L$ and the creatinine clearance rate reached $58.2 \pm 15.6 \text{mL/min per } 1.73 \text{m}^2$. The mean serum level of $\beta_2$-microglobulin also decreased from $37.8 \pm 7.6 \mu M/L$ to $4.05 \pm 0.6 \mu M/L$ (upper normal limit, $3 \mu M/L$). Rheumatological complaints improved substantially: shoulder stiffness, which was observed in 12 patients, totally disappeared in eight and markedly improved in the remaining four patients. The clinical score decreased from $2.47 \pm 1.8$ to $0.35 \pm 0.7$ during transplantation ($P < 0.0005$). In one patient in whom the surgical procedure for CTS was scheduled before transplantation, pain totally disappeared with transplantation and surgery was no longer indicated. In the majority of patients studied, symptoms disappeared within 24 h after transplantation, suggesting that factors other than improvement in renal function may play a role.

Long-Term Follow-Up

Patients were distributed among two groups according to their renal graft outcome: Group I included those patients with ongoing satisfactory renal function ($N = 10$; mean follow-up period was 63.1 ± 36.5 months) and Group II was constituted of those patients with subsequent graft failure and who returned to hemodialysis ($N = 7$; mean duration of graft function was 47.4 ± 39 months). These two groups were similar for age and sex distribution, as well as for the mean duration of hemodialysis before transplantation, and they were submitted to the same immunosuppressive protocol. As expected following the distribution criteria, the number of rejection episodes per patient was significantly lower in Group I when compared with Group II ($0.2 \pm 0.42$ versus $1.28 \pm 1.25$; $P < 0.03$).

Group I. The ten patients included in this group had a mean follow-up period of 63.1 months (range, 22 to 133 months). Eight of them had no rejection episodes and two experienced a single mild reversible acute rejection. Mean serum creatinine concentration was $160 \pm 35 \mu M/L$ (range, 106 to 220 $\mu M/L$) at the last follow-up examination. Two patients have chronic rejection (proteinuria, mild hypertension) with satisfactory renal function (serum creatinine levels, 119 and 220 $\mu M/L$). The serum level of $\beta_2$-microglobulin was $3.8 \pm 0.9 \mu M/L$.

All patients were operated on for uni- or bilateral CTS before transplantation, but none needed surgery after the transplantation was performed. The clinical improvement observed during the early period after transplantation persisted throughout the follow-up period. It was observed despite the reduction in immunosuppressive therapy: steroid doses were decreased to either 5 to 10 mg/day or 20 mg every other day, and azathioprine treatment (in two patients) and cyclosporine (in eight patients) was reduced as scheduled, aiming for a target whole-blood through level of 100 ng/mL. A mean of 12 ± 7.5 (range, 2 to 24) bone cysts were recorded at the time of transplantation. This number remained remarkably constant throughout the follow-up period (12.1 ± 7.7 at the last oupa-

<table>
<thead>
<tr>
<th>TABLE 2. Evolution of DRA after transplantation in patients with long-term satisfactory graft function (Group I)$^a$</th>
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<tbody>
<tr>
<td>Patient</td>
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<tr>
<td>---------</td>
</tr>
<tr>
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<tr>
<td>9</td>
</tr>
<tr>
<td>10</td>
</tr>
<tr>
<td>Mean±SD</td>
</tr>
</tbody>
</table>

$^a$ Wilcoxon’s test for paired data was applied to compare before versus during transplantation.

$^b$ Dose administered every other day.

$^c$ $P < 0.002$.

$^d$ $P < 0.008$.

$^e$ $P < 0.001$.  

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Figure 1. Evolution of a humeral bone cyst in one patient at time of transplantation (A) and 10 yr after satisfactory graft function (B). Note the remarkable stability of the lesion despite prolonged normal renal function.

tient visit) (Table 2). Besides the number of cysts, their size did not change during the transplantation period, suggesting that despite satisfactory renal function, there is no progression nor regression of the lesions (Figure 1). In one patient with hip cysts, hip fracture occurred and a femoral prosthesis was inserted 2 yr after transplantation. The material that was removed fulfilled the criteria for amyloidosis and contained β2-microglobulin.

Group II. The seven patients included in this group had a mean follow-up period after transplantation of 47.4 ± 39 months and five of them had experienced more than one episode of acute rejection (Table 3). Six patients eventually lost their grafts because of chronic rejection and one because of recurrence of membrano-proliferative glomerulonephritis. All of them had reached a satisfactory renal function (serum creatinine concentration <200 μmol/L) within 1 month after transplantation.

During transplantation, the Group II patients experienced the same improvement in DRA symptoms as the patients included in Group I. No patient had to be operated on for CTS, and shoulder stiffness was reduced in all of them; regular monitoring of x-rays did not show any new cystic bone lesion. In contrast, after returning to hemodialysis, β2-microglobulin amyloidosis symptoms reappeared early and frequently overcame those observed before transplantation. In fact, five out of the seven patients had to be operated or reoperated on for CTS after 16.7 ± 12 months. All of the specimens surgically obtained were positive for β2-microglobulin amyloidosis. Three patients necessitated surgery within the first yr after reinstitution of hemodialysis (3, 6, and 12 months, respectively). Shoulder stiffness, markedly disabling in some patients, reappeared as soon as hemodialysis sessions recommenced. The clinical score significantly increased from 0.71 ± 0.95 to 3.86 ± 1.46 (P < 0.0313). The number of cysts significantly increased from 17.4 ± 11.9 before transplantation to 21 ± 11.6 at the last follow-up examination (Figure 2).

As shown in Table 1, in our population, the mean interval between dialysis onset and DRA diagnosis during the first dialysis period before transplantation was 130 months. During the second dialysis period, joint pain suggesting clinical recurrence of DRA was observed within the first dialysis sessions after graft failure in all of the patients and DRA was histologically proven in five out of the seven included patients within 30 months. Therefore, a mean transplantation period of 44 months failed to cure the amyloid process as it did not reset the delay of having DRA to the initial 130 months.

DISCUSSION

The study presented here shows that DRA symptoms are remarkably improved by transplantation.

<table>
<thead>
<tr>
<th>Patient Number</th>
<th>Duration of TX (months)</th>
<th>HD Follow-Up After TX (months)</th>
<th>Current Status</th>
<th>Number of Surgical Procedures for CTS</th>
<th>Intensity of Shoulder Stiffness</th>
<th>Number of Bone Cysts</th>
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<tr>
<td>11</td>
<td>24</td>
<td>114</td>
<td>HDF</td>
<td>Before TX 4 2 4/71</td>
<td>After Resuming HD 4</td>
<td>Before TX 8 8 8</td>
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<td>12</td>
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<tr>
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<td>120</td>
<td>36</td>
<td>Re-TX</td>
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<td>0 1 3 29 12 22</td>
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</tr>
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<td>15</td>
<td>50</td>
<td>54</td>
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<td>3 3 25 24 25</td>
<td>25</td>
</tr>
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<td>40</td>
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<td>0 0</td>
<td>2 2 21 21 21</td>
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<td>20</td>
<td>Death</td>
<td>2 2 12</td>
<td>4 4 31 31 31</td>
<td>32</td>
</tr>
</tbody>
</table>

Mean ± SD 47.4 ± 39 44.1 ± 31 1.4 ± 1 1.1 ± 0.9 16.7 ± 12 2.28 ± 2.14 0.71 ± 0.95 2.86 ± 1.46b 17.4 ± 11 17.3 ± 11 21 ± 11c,d

a CTS, carpal tunnel syndrome; TX, transplantation; HD, hemodialysis; HDF, hemodiafiltration.
b P < 0.030 (during TX versus after resuming HD).
c P < 0.016 (during TX versus after resuming HD).
d P < 0.031 (before TX versus after resuming HD).
Furthermore, it also shows that the group of patients with transplant failure, even after a long period of normal graft function, had clinical manifestations of DRA surprisingly early after resuming dialysis. In fact, there was no objective appearance of new lesions or progression of the preexisting ones during the transplantation period, but successful transplantation was unable to reset the amyloidogenic processes at the level that they had been when the patients started dialysis for the first time. The delay of appearance of the disease was strikingly shorter during the second dialysis period (17 months versus 130 months). These findings suggest that although the clinical expression of DRA is arrested, the pathological processes underlying it are unlikely to be reversed during transplantation.

Several factors could participate in the early disappearance of pain. Anesthesia could have a role during the immediate period after surgery. Over the long term, immunosuppressive treatment (particularly steroids) and withdrawal of dialysis are more likely to prevent clinical manifestations. Steroids have traditionally been used to relieve pain in chronic inflammatory processes unrelated to dialysis, with a lower incidence of gastric side effects than nonsteroidal anti-inflammatory agents (12). As an extension of this approach, they have also been proposed for use in DRA (13). In a study conducted by Bardin, low-dose prednisolone (4 to 8 mg each morning) was effective in resolving DRA symptoms. However, 12 out of 27 patients were withdrawn from the study because of death (seven of 12) or side effects (five of 12), leading the author to be most cautious in the indications of this treatment (13). The doses we used in our study were equally low, as most of the patients in Group I are receiving 10 mg a day or 20 mg every other day and they do not have clinical symptoms of DRA. A controlled study comparing the effects of low-dose steroids in transplant and hemodialysis patients with DRA may help to clarify this issue. Finally, Campistol et al. reported that two patients receiving CsA monotherapy had improved DRA symptoms (9), suggesting that steroids are not the only factor responsible for the clinical improvement of DRA in transplant patients.

Among the other factors, withdrawal of dialysis might be an important one. In the pathogenesis of DRA, it is likely that the activation of membrane-associated bioincompatibility mechanisms in a regular and protracted manner would favor the appearance of β2-microglobulin amyloid fibrils. Extending this view, some authors have reported that the less bioincompatible membranes would delay or prevent patients from DRA (5,14). Many investigators have determined the serum levels of β2-microglobulin in different populations, separated according to the membrane that they use. Some of these reports suggest that cellulose membranes would increase the synthesis of β2-microglobulin (15,16), whereas others have suggested the opposite (17). It has been suggested that the increase in IL-1 or the complement activation induced by some dialysis membranes would result in an increase in the synthesis of β2-microglobulin (16), but this point remains controversial (18). Finally, even if the increase in the serum level of β2-microglobulin was confirmed, there seems to be agreement that the serum level of β2-microglobulin is not correlated with the appearance of β2-microglobulin amyloidosis and it cannot be taken as a good marker to predict the disease (19). Further, it is to be stressed that β2-microglobulin amyloidosis has also been reported in peritoneal dialysis patients (20), as well as in patients with chronic renal failure, before starting any renal replacement therapy (21). These reports suggest that protracted chronic renal failure is of crucial importance in the development of β2-microglobulin amyloidosis. Transplantation is the only treatment that avoids this main factor, because it reestablishes satisfactory renal function.

Beyond the clinical impact of renal transplantation on DRA, our study aimed to assess the variation of DRA lesions. Although we have commented on the lack of correlation between the serum level of β2-microglobulin and the risk of developing DRA, nothing is known about the serum levels of β2-microglobulin in the supposed maintenance of the disease once it exists. The β2-microglobulin serum level was dramatically improved by transplantation, but it was not within the normal range, as the creatinine clearance rates of these patients were significantly lower than normal (58.2 ± 15.6 mL/min per 1.73 m²). Two observations are relevant to this point; the first is the presence of β2-microglobulin in light-chain amyloid fibrils in a patient with normal serum and synovial levels of β2-microglobulin (22). The second is that the serum level of β2-microglobulin in a population of uninephrectomized kidney donors that we have stud-
ied (3.05 ± 1.9 mg/L for a technetium-99m-Diamine triethylene pantamic acid (DTPA) clearance of 70.5 ± 20 mL/min per 1.73 m²) (23) is higher than that observed in healthy volunteers (1.67 ± 0.05 mg/L for a DTPA clearance of 109.5 ± 1.6 [N = 127]). Despite long follow-up periods of these populations of kidney donors, no β2-microglobulin amyloidosis has been reported, suggesting that a slight increase in the serum level of β2-microglobulin is not sufficient to induce DRA. When analyzing it from the opposite side, from our x-ray data, it would seem that a near normalisation of the β2-microglobulin serum level in transplanted patients is not enough to allow β2-microglobulin amyloid deposits to be resorbed.

Traditionally, amyloid has been considered non-resorbable in vivo; this is thought to be a result of its β-pleated fibrillar structure (24). However, Hawkins and Pepys' group, using a gammagraphic scan of radiolabeled amyloid P component, has suggested a regression of amyloid deposits in AA amyloidosis (25). There are a few reports suggesting such a regression of amyloid lesions in vivo, which have not been widely confirmed (26–29). In vitro, Skogen and Natvig have succeeded in degrading amyloid deposits in the presence of proteases in AA amyloidosis (30). Finally, we have also been successful in solubilizing some of the protein components of β2-microglobulin amyloidosis in vitro very recently (Garcia-Garcia M, et al: "In vitro" resuspension of proteins from β2-microglobulin amyloid deposits [Abstract], Eur Dial Transplant Assoc, 1995).

One of the characteristic features of β2-microglobulin amyloidosis is its predilection for bone and tendons (1–4). Ultrasound studies have been proposed to assess the evolution of the tendinous lesions (31). Unfortunately, we were unable to perform a reliable assessment of the amyloid lesion by this technique in our group and we therefore limited our study to x-ray follow-up examinations of the DRA lesions. We found no variation in the number or the size of DRA lesions over the transplantation period; there was no progression or regression. In keeping with previous reports (8,32), our results do not seem to confirm the possibility of resorption of amyloid deposits in vivo, as proposed by Hawkins and Pepys for AA amyloidosis (25). Regardless of the x-ray assessment during the transplantation period, our study also assessed for the first time the delay in the recurrence of clinical amyloidosis after transplantation. Interestingly, the patients with graft failure (Group II) had to undergo surgery for DRA remarkably early after resuming dialysis. These results, although indirect, clearly suggest that amyloidosis was still and already present when patients were switched back to dialysis, a big rebound in amyloidogenesis with a relapse in DRA after transplantation being highly unlikely. The inflammatory syndrome linked to chronic allograft rejection, the decrease and/or the withdrawal of immunosuppressive drugs, associated with membrane-induced bioincompatibility phenomena may also participate in the accelerated recurrence of DRA symptoms. However, their roles remain to be defined.

In summary, by taking DRA and successful transplantation as the entrance criteria and ensuring a long-term follow-up, our study resulted in an A-B-A design (A and B being hemodialysis and transplantation periods, respectively). This allowed us to assess three different aspects of DRA: (1) transplantation as the proposed treatment for DRA; (2) the possible resorption of the deposits once they are formed; and (3) the natural history and final outcome of DRA with the treatment presently available (renal replacement therapy and kidney transplantation). Our data show that transplantation represents a good treatment to arrest the evolution of the symptoms of DRA, but it does not seem to cure it. The regression of amyloid deposits is not supported by our data. DRA remains clinically silent but present during transplantation and ready to relapse after graft failure, thus stressing the importance of investigating the mechanisms of the genesis of amyloidosis in general and not simply its treatment once it is present. Until a prophylaxis for β2-microglobulin amyloidosis is available, we propose transplantation as an early treatment of end-stage renal failure, if possible, before DRA occurs. This approach is the one that will certainly postpone this crippling complication for a longer period of time.

ACKNOWLEDGMENTS

We thank Dr. Peter G. Kerr for his knowledgeable discussions. The research project of Dr. Argilés is partially funded through a grant from the Extramural Grant Program of Baxter Healthcare Corporation (McGaw Park, IL). Funding was also received from the Centre Hospitalier Universitaire de Montpellier.

REFERENCES