Amniotic Membrane With Living Related Corneal Limbal/Conjunctival Allograft for Ocular Surface Reconstruction in Stevens-Johnson Syndrome

José A. P. Gomes, MD; Myrna S. Santos, MD; Ângela S. Ventura, MD; Waleska B. C. Donato, MD; Marcelo C. Cunha, MD; Ana Luisa Höfling-Lima, MD

Objective: To report the use of preserved amniotic membrane and living related corneal limbal/conjunctival transplantation in total limbal stem cell and conjunctival deficiency secondary to severe Stevens-Johnson syndrome.

Design: Prospective, noncomparative, interventional case series.

Patients and Methods: Ten eyes of 10 patients with total limbal stem cell and conjunctival deficiency secondary to Stevens-Johnson syndrome underwent excision of cicatricial tissue followed by amniotic membrane and living related corneal limbal/conjunctival transplantation.

Main Outcome Measures: Reconstruction of corneal epithelium (clear appearance without epithelial defect, normal fluorescein permeability, and the absence of conjunctiva-derived goblet cells on impression cytologic testing), decrease in corneal vascularity, and improvement in visual acuity.

Results: During a mean follow-up of 16.7 months, satisfactory ocular surface reconstruction was obtained in 2 eyes (20%), with reduced inflammation and vascularization and a mean epithelialization time of 3 weeks. Surgical failure was observed in 4 cases (40%) and complications (infection) in 4 cases (40%). Visual acuity improved in 4 eyes (40%), remained stable in 5 eyes (50%), and decreased in 1 eye (10%).

Conclusions: Amniotic membrane and living related corneal limbal/conjunctival transplantation were successful in 20% of severe cases of total limbal stem cell and conjunctival deficiency secondary to Stevens-Johnson syndrome. A high proportion of postoperative complications, in particular, infection, seemed to jeopardize a favorable outcome.

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STEVENS-JOHNSON syndrome (SJS) can be defined as an inflammatory disease that involves the skin and at least 2 mucous membranes. It is possibly an autoimmune phenomenon related to medications and/or infections.1,2 It is considered part of the erythema multiforme (EM) spectrum, which comprises EM minor (skin and, rarely, one mucous membrane involvement), SJS or EM major, and toxic epidermal necrolysis or Lyell syndrome, which is the most severe form of EM, characterized by extensive epidermal loss.

The acute stage of the ocular disease is characterized by bilateral catarrhal and membranous conjunctivitis.1 During the chronic stage of the disease, most patients have various alterations of the ocular surface, such as symblepharon, entropion, trichiasis, dry eye, limbal stem cell deficiency, conjunctival inflammation, and corneal neovascularization.1,3 Destruction of limbal stem cells is due to prolonged inflammation and results in replacement of the corneal epithelium by conjunctival epithelium (conjunctivalization).3 In cases associated with severe dry eye, the pattern of relapsing inflammation induces squamous metaplasia and keratinization.3

The treatment of the chronic phase aims at restoration of the anatomical structures and physiologic properties of the ocular surface, thereby restoring the corneal and conjunctival epithelium.4 Limbal transplantation alone may restore normal corneal epithelium.5,6 Reconstruction of the conjunctival epithelium may be achieved by substitution of other mucosal tissue for the affected conjunctiva.7 The stability of the restored ocular surface must also be maintained by restitution of an appropriate biological microenvironment.3

The amniotic membrane (AM), the inner part of the placenta, consists of a thick basement membrane of collagen type IV and laminin, and an avascular stroma.6 It has important properties, such as the induction of
migration and adhesion of epithelial cells, inhibition of inflammatory, angiogenic, and scarring reactions, and is considered immunologically inert. 3-7 During the last several years, preserved AM has been used for the reconstruction of the ocular surface in various situations, such as cicatrical processes, epithelial defects, pterygium, symblepharon, and bullous keratopathy. 3,6,8-10 The transplantation of AM associated with a limbal graft, mainly derived from cadaver eyes, has been used in the treatment of SJS, with varying outcomes. 3,9

The purpose of this study was to investigate the surgical outcome of AM transplantation associated with limbal/conjunctival allografts derived from living related donors (lr-CLAL) in a series of patients with total limbal stem cell and conjunctival deficiency secondary to Stevens-Johnson syndrome.

PATIENTS

Ten eyes of 10 consecutive patients with total limbal stem cell and conjunctival deficiency secondary to SJS were included in this study, which was approved by the ethics committee of the Department of Ophthalmology, Federal University of São Paulo, Brazil, and followed the tenets of the Declaration of Helsinki. There were 7 male (70%) and 3 female (30%) patients, with an average age of 30.5 years (age range, 9-64 years).

The diagnosis of SJS was based on a history of severe inflammation of the oral, dermal, and tracheal mucous membranes after taking medicine or having an infection. The initial clinical evaluation consisted of slitlamp biomicroscopy to examine the eyelid, conjunctiva, fornices, and the cornea. Total limbal stem cell deficiency was diagnosed clinically by the presence of conjunctivalization of the entire corneal surface, vascularization, chronic inflammation, and recurrent or persistent corneal epithelial defect. All patients had severe dry eye syndrome with values less than 5 mm on the Schirmer test I.

Individuals were treated at the Department of Ophthalmology, Federal University of São Paulo, each having given their written informed consent to undergo the surgical procedure. The clinical data of the patients are presented in Table 1.

PREPARATION AND PRESERVATION OF AM

Human AM was prepared and preserved using the method proposed by Kim and Tseng, 7 with some modifications. Human placenta was obtained at the time of cesarean section after obtaining oral informed consent from donors who had negative serologic tests for hepatitis B and C virus, syphilis, and human immunodeficiency virus. Under sterile conditions, the amnion was separated from the chorion by blunt dissection and washed with a phosphate-buffered saline solution containing 1000 U/mL of penicillin, 20 µg/mL of streptomycin, and 2.5 µg/mL of amphotericin B. Under a laminar flow air hood, the amnion was flattened onto nitrocellulose paper, with the epithelium–basement membrane surface up. The paper with the adherent membrane was then cut into pieces of 3 × 3 and 4 × 4 cm and stored at −80°C in a sterile vial containing glycerol and Dulbecco’s Modified Eagle Medium (1:1).

SURGICAL PROCEDURE

Lacrimal punctal occlusion by electrocauterization was performed in all patients in whom the punctum was not already occluded owing to the cicatrical disease process or previous cautery. Surgical correction of trichiasis, distichiasis, and symblepharon was performed prior to lr-CLAL AM transplantation. Symblepharon was corrected with oral mucosal transplantation, and trichiasis and distichiasis were corrected with epilation.

Except for 3 patients who received general anesthesia, all surgeries were performed with peribulbar anesthesia using 2% lidocaine and 0.5% maraine. First, the conjunctival epithelium with fibrous tissue covering the cornea was removed to approximately 3 to 4 mm posterior to the limbus. After defrosting the tissue, the AM was removed from the storage medium, peeled off the nitrocellulose paper, transferred to the recipient’s eye, and fitted to cover the entire defect, with the basement membrane in the upside position. The membrane was attached to the episclera and conjunctiva with a 9-0 nylon running suture.

The donor selection for lr-CLAL was based on HLA I and II typing of all first-degree relatives, and we chose the donor who best matched the patient. If the limbal donor had more than 75% compatibility, we did not start systemic immunosuppression. After topical anesthesia, a 5-mm-wide strip of bulbar conjunctiva from the 2- to 10-o’clock position was cut using microscissors. The limbal area was exposed and a lamellar dissection of 0.5 mm of the corneal limbus was performed using a surgical blade. The central side of the graft was cut with Vannas scissors. The conjunctival-limbal graft was divided into 2 rectangular pieces, which were transferred to the 12- and 6-o’clock positions of the affected eye and sutured through the AM to the episclera/limbus with a 10-0 nylon suture in each corner. After the lr-CLAL AM transplantation, a lateral tarsorrhaphy was performed in all cases to prevent desiccation. When necessary, penetrating keratoplasty (PKP) or lamellar keratoplasty, with or without anterior segment reconstruction with cataract extraction and intraocular lens insertion, were performed simultaneously.

POSTOPERATIVE TREATMENT

During the postoperative period, topical 0.1% dexamethasone and 0.3% ofloxacin were used 5 times daily for the first month and progressively tapered. Patients were also instructed to use preservative-free artificial tears every hour and to wear special dry-eye glasses. The most severe cases (Schirmer I=0 mm and/or delayed epithelialization) were also instructed to use 50% diluted (with balanced salt solution) autologous serum drops.

In the cases of lr-CLAL with HLA compatibility less than 75%, or when corneal transplantation was performed, systemic immunosuppression was started with 1 mg/kg of oral prednisone daily and 5 mg/kg of cyclosporine A. The blood target for cyclosporine A was 100 to 150 ng/mL. Oral prednisone was progressively tapered and discontinued after 8 weeks. The cyclosporine dose was tapered to 2 to 4 mg/kg in 2 to 4 weeks and continued indefinitely. Serum creatinine was monitored monthly, and any significant increase in serum creatinine levels were managed by decreasing the dose. Blood pressure, complete blood cell count, blood glucose levels, and liver functions were also evaluated monthly. In patients who demonstrated inflammation on tapering of oral immunosuppression, appropriate levels of systemic immunosuppressive agents were maintained. When immunologic rejection of the allograft developed, 1% topical prednisolone acetate was given every 1 to 2 hours, and systemic immunosuppression was increased.

Ophthalmologic evaluation was performed in all patients weekly for the first 2 months and then twice per month until the sixth month of follow-up, after which they were evaluated monthly. After the fifth month, impression cytologic analysis was performed whenever possible. 5

Successful ocular surface reconstruction was defined on the basis of corneal epithelialization, decrease in corneal neovascularization, and improvement in visual acuity. 3 Corneal ep-
The average time between the onset of the disease and the surgical intervention was 11.5 years (range, 1.5-40 years). The mean follow-up time was 16.7 months (range, 1.5-40 months) and the mean time to epithelialization was 11.5 years (range, 1.5-40 years). The mean follow-up time was 16.7 months (range, 1.5-40 months) and the mean time to epithelialization was 11.5 years (range, 1.5-40 years).

RESULTS

Surgical success was observed in 2 cases (20%), failure in 4 cases (40%), and complications (infection) in 4 cases (40%) (Figure 1 and Figure 2). One case of failure (case 10) developed a granulomatous reaction and rejection, and 1 case developed an acute inflammatory reaction with corneal melting and perforation (case 3). Infection occurred during the early postoperative period (<1 month) in 3 cases and in the late postoperative period (11 months) in 1 case (case 6). All of these patients were systemically immunosuppressed. Case 2 had a mixed infection with *Acanthamoeba*, *Candida* species, and *Enterobacter* species. The infection in case 8 was caused by the *Candida* species, case 1 by *Staphylococcus epidermidis*, and case 6 by *Pseudomonas aeruginosa*. The infection was controlled in all cases with appropriate antibacterial/parasitic/fungal medication. The corneas of all of these cases developed opacification, neovascularization, and conjunctivalization. The Kaplan-Meier survival curve shows that reconstruction failed in 5 cases (50%) in the first month and in 3 more cases (30%) 1 year after the surgery (Figure 3). After the last follow-up visit, we found improvement of visual acuity in 4 patients (40%), the preoperative acuity remained unchanged in 5 (50%), and a decrease in vision was ob-

### Table 1. Preoperative Data of Patients With Cicatricial Keratoconjunctivitis Secondary to SJS

<table>
<thead>
<tr>
<th>Patient/ Sex/ Age, y</th>
<th>Age at Onset of SJS, y</th>
<th>Treatment (Before)</th>
<th>Limbal Deficiency</th>
<th>Stroma</th>
<th>Schirmer Test I</th>
<th>Fluorescein</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/M/45 4</td>
<td>Ir-CLAL, MT, LKP</td>
<td>Total</td>
<td>Th + V</td>
<td>0</td>
<td>ED + Pk + K</td>
<td></td>
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<tr>
<td>2/M/64 40</td>
<td>PKP, MT</td>
<td>Total</td>
<td>SN + V</td>
<td>0</td>
<td>ED + K</td>
<td></td>
</tr>
<tr>
<td>3/F/9 2</td>
<td>2 PKPs</td>
<td>Total</td>
<td>SN + V</td>
<td>0</td>
<td>ED + Pk + S</td>
<td></td>
</tr>
<tr>
<td>4/M/14 10</td>
<td></td>
<td>Total</td>
<td>Th + V</td>
<td>&lt;5 mm</td>
<td>Pk</td>
<td></td>
</tr>
<tr>
<td>5/F/33 8</td>
<td>MT</td>
<td>Total</td>
<td>Th + V</td>
<td>&lt;5 mm</td>
<td>Pk</td>
<td></td>
</tr>
<tr>
<td>6/M/62 11</td>
<td></td>
<td>Total</td>
<td>Th + V</td>
<td>&lt;5 mm</td>
<td>Pk</td>
<td></td>
</tr>
<tr>
<td>7/M/12 1.5</td>
<td>MT, AM</td>
<td>Total</td>
<td>Th + V</td>
<td>&lt;5 mm</td>
<td>Pk</td>
<td></td>
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<tr>
<td>8/M/20 15</td>
<td></td>
<td>Total</td>
<td>Th + V</td>
<td>&lt;5 mm</td>
<td>Pk</td>
<td></td>
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<tr>
<td>9/M/27 8</td>
<td>Conjunctival flap</td>
<td>Total</td>
<td>Th + V</td>
<td>&lt;5 mm</td>
<td>Pk</td>
<td></td>
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<tr>
<td>10/M/18 16</td>
<td></td>
<td>Total</td>
<td>Th + V</td>
<td>&lt;5 mm</td>
<td>Pk + K</td>
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</tr>
</tbody>
</table>

### Table 2. Surgical Outcome After Reconstruction of the Ocular Surface With Amniotic Membrane Transplantation Associated With Ir-CLAL in Patients With Stevens-Johnson Syndrome

<table>
<thead>
<tr>
<th>VA Before</th>
<th>VA After</th>
<th>Associated Procedure</th>
<th>Systemic Immunosuppression</th>
<th>Follow-up, mo</th>
<th>Epithelialization Time, wk</th>
<th>Fluorescein</th>
<th>Results</th>
<th>Time of Failure, mo</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 HM CF, 50 cm PkPs, AMTpl Yes 22 2 Pk C (early infection) 11 (3 wk after AMTpl)</td>
<td></td>
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<td></td>
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<tr>
<td>2 HM NLP PKPs, ECCE, IOL Yes 14 3 ED C (early infection) 1</td>
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<tr>
<td>3 HM PKPs Yes 18 4 ED F (acute inflammation and melting) 1</td>
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<tr>
<td>4 HM PKPs, ECCE Yes 24 3 Pk Successful</td>
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<td></td>
<td></td>
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<tr>
<td>5 HM PKPs, ECCE Yes 15 3 ED F (chronic inflammation) 3</td>
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<tr>
<td>6 HM PKPI Yes (after PKP) 19 3 Pk C (late infection) 12</td>
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<td>7 HM ... Yes 19 3 Pk F (chronic inflammation) 12</td>
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<tr>
<td>8 HM CF, 2m ... Yes 12 3 ED, melting Pk F (chronic inflammation) 1</td>
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<tr>
<td>9 HM CF, 2m ... Yes 12 4 Pk Successful</td>
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<tr>
<td>10 HM ... Yes 12 4 ED C (granulomatous reaction and rejection) 2</td>
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<td></td>
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</table>

Abbreviations: AM, amniotic membrane; AMTpl, amniotic membrane transplantation for partial limbal deficiency in a latter stage; C, complication; CF, counting fingers; ECCE, extracapsular cataract extraction; ED, epithelial defect; f, failure; HM, hand movement; IOL, intraocular lens with implantation of an intraocular lens; Ir-CLAL, living related limbal/conjunctival allograft; LKP, lamellar keratoplasty; NLP, no light perception; Pk, punctate keratopathy; PKP, penetrating keratoplasty performed in a latter stage; PKPs, simultaneous penetrating keratoplasties; VA, visual acuity; ellipses, not applicable.
served in 1 patient (10%). The postoperative results are presented in Table 2.

**COMMENT**

Ocular involvement in SJS frequently results in a combination of clinical manifestations, such as limbal dysfunction, severe dry eye, and cicatricial alterations of the conjunctiva and eyelids. Because of the combination of various alterations, the treatment of this disorder is complex and difficult. Therefore, until recently, keratoprosthesis has been considered the only therapeutic option to restore visual acuity in these cases.3

Several surgical approaches for the reconstruction of the ocular surface in cicatricial disorders with limbal deficiency have been tried. In 1995, Kim and Tseng7 re-

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**Figure 1.** A, The preoperative appearance of case 1 shows total limbal stem cell and conjunctival deficiency with conjunctivalization and inferior keratinization. B, The postoperative appearance 6 months after limbal/conjunctival allografts derived from living related donors and amniotic membrane (AM) transplantation and penetrating keratoplasty shows corneal transparency with a stable corneal surface. C, The postoperative appearance of case 1 after 10 months. Observe the decrease in corneal epithelial regularity and the recurrence of fibrovascular proliferation over the inferior half of the cornea. D, Bacterial ulcer and failure of ocular surface reconstruction after inferior pannus resection and AM transplantation (12 months after the initial surgery). A and B are published with the permission of Editora Cultura Medica, Rio de Janeiro, Brazil.

**Figure 2.** A, Postoperative appearance 3 weeks after limbal/conjunctival allografts derived from living related donors and amniotic membrane Ir-CLAL AM transplantation (case 10). Note the granulomatous reaction over the entire ocular surface. B, Postoperative appearance 12 months after Ir-CLAL AM transplantation (case 7) shows recurrence of conjunctivalization with superficial neovascularization and epithelial defect.
introduced the use of AM in an experimental model of limbal deficiency. Since then, various studies using AM transplantation with or without limbal transplantation have been done for the treatment of ocular surface diseases, with satisfactory results and corneal epithelialization ranging from 41% to 100%.3,4,9,11,13,17,18

The present study included 10 patients with severe limbal deficiency and dry eye, as well as important cicatricial alterations of the eyelids secondary to SJS. Our therapeutic strategy for the reconstruction of the ocular surface consisted of AM transplantation associated with Ir-CLAL. Moreover, we added measures for the treatment of dry eye syndrome, such as preservative-free artificial tears, topical autologous serum, lacrimal punctum occlusion, and tarsorrhaphy.

Satisfactory reconstruction of the ocular surface after a mean follow-up of 16.7 months was observed in only 2 cases (20%). These results were less impressive than those reported by Tsubota et al., who achieved a success rate of 85.5% in cases of advanced SJS and ocular cicatricial pemphigoid after a mean follow-up of 5 months. However, our results were consistent with those of a larger series of SJS and ocular cicatricial pemphigoid with a longer follow-up (mean, 3 years) performed by Tsubota et al. This difference may be explained by a dramatic reduction of the surgical success rate with a longer follow-up in severe cases. In a recent report, Solomon et al. also observed a progressive decline of keratolimbal allograft (KLAL) survival for total limbal stem cell and conjunctival deficiency secondary to a number of different severe ocular surface diseases. Although the 2-year survival of KLAL for SJS was encouraging (62%), none of the patients with SJS who received combined PKP had a clear graft after 2 years of follow-up.17

The surgical success obtained in our series with SJS was lower than in patients with ocular burn (approximately 60%-70%).18 This was possibly due to the more accentuated instability of the ocular surface (severe dry eye, keratinization, eyelid problems) and the associated immunologic alterations in SJS. In our study, ocular surface reconstruction failed in all patients with Schirmer test scores equal to zero. A similar finding was reported by Shimazaki et al19 in patients with SJS who underwent KLAL associated with AM transplantation. Tear film function evaluation should be considered as an important prognostic factor in patients with SJS who are undergoing ocular surface reconstruction.

Important causes of limbal transplantation failure include rejection, acute inflammatory reaction, and persistent inflammation.20 An acute inflammatory reaction type of failure occurs within the first 2 weeks following the limbal transplantation.21 We observed 2 different types of acute reaction: one was related to corneal melting and perforation in a child who had stromal necrosis at the time of the surgery, and the other to a granulomatous reaction. Immunologic rejection usually occurs 2 months after the stem cell transplantation and was diagnosed in only 1 case in our series. Patients with rejection have symptoms of pain and photophobia and signs of vasoconstriction and edema at the limbus and graft-host interface, superficial keratopathy and, in some cases, an epithelial rejection line may be observed.21,22 In our case, an increase in oral and topical immunosuppression did not control the rejection. Chronic nonimmunologic inflammation is another important cause of limbal transplantation failure and was observed in 2 cases in our series.22 It is usually associated with severe tear film dysfunction and keratinization and can compromise the survival of stem cells transplanted and increase the risk of rejection.21

We observed a high number of postoperative complications in the SJS group, notably, infection (40%). Similarly, Samson et al. reported a high incidence of infection in chronic immunoinflammatory eye diseases in patients who underwent limbal stem cell transplantation. In patients with SJS, the instability of the ocular surface, which can be demonstrated by the presence of epitheliopathy associated with severe dry eye, eyelid margin/lash problems, use of systemic immunosuppression, and immunologic alterations due to SJS, may explain these results. All of our patients who developed postoperative infection were covered by broad-spectrum topical antibiotics, mostly, ofloxacin. In unpublished data by 3 of us (M.S.S., A.L.H.-L., J.A.P.G, along with Catia R. T. Valadares, MD; Maria Cecilia Z. Yu, MSC; 2001), an extremely high number of pathogenic bacteria was found in conjunctival scrapings of the eyes of patients with SJS. Culture of the conjunctiva prior to the surgery and prophylactic use of appropriate topical antibiotic may benefit the outcome of ocular surface reconstruction in SJS.

Despite the promising results of AM transplantation as adjuvant therapy associated with limbal transplantation in patients with cicatricial keratoconjunctivitis, it remains unclear if it is better than limbal transplantation alone. Furthermore, there is no consensus regarding the difference in epithelial stability after transplantation of limbal grafts derived from either cadaver or living donors. An advantage of KLAL is the greater amount of tissue available. The advantage of Ir-CLAL is the provision of con-
junctional epithelial and goblet cells—of particular importance in cases of severe dry eye—as well as the possibility of performing HLA matching, which may make systemic immunosuppression unnecessary in cases with totally compatible donors. At least it can decrease limbal graft rejection when systemic immunosuppression is decreased in cases with incomplete HLA matching. Kwitko et al performed a prospective study of allogeneic conjunctival transplantation in 12 cases of bilateral ocular surface disorders, and reported fewer rejection episodes and a better survival in 12 cases of bilateral ocular surface disorders compared with non-identical (50% identity) HLA-matched conjunctival allografts, and reported fewer rejection episodes and a better survival.17 Our experience with evident, to the point at which none of the grafts survived after 5 years of follow-up.17 Our study found risk of infection to be an additional important factor. Additional prospective studies with larger numbers of patients and more prolonged follow-up will yield more information on the nature and treatment of this problem.

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Corresponding author and reprints: Jose A. P. Gomes, MD, R Sabará, 566 Cjuto 212, 01239-010 São Paulo, Brazil (e-mail: japgomes@uol.com.br).

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