Transplant International ISSN 0934-0874

ORIGINAL ARTICLE

Marked erythrocyte microcytosis under primary immunosuppression with sirolimus

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Keywords
anemia, erythrocyte microcytosis, mycophenolate mofetil, sirolimus

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Received: 15 March 2005
Revision requested: 5 April 2005
Accepted: 9 June 2005

doi:10.1111/j.1432-2277.2005.00190.x

Summary

The preliminary observation of marked erythrocyte microcytosis in patients treated with sirolimus (SRL) and mycophenolate mofetil (MMF) has been evaluated as part of a prospective study comparing SRL and cyclosporin A (CsA) as a primary immunosuppressant. Normal risk *de novo* kidney recipients were randomized either to SRL or to CsA. Additional immunosuppressants consisted of MMF and prednisone. In patients with erythrocyte microcytosis, iron deficiency was excluded by measuring serum ferritin and transferrin saturation rate. Fifty-nine patients (30 in SRL and 29 in CsA) were included. Mean corpuscular volume (MCV) (fl) on day 7 was 91.7 ± 4.8 in SRL group versus 91.4 ± 4.2 in CsA group ($P = 0.77$), whereas mean MCV on day 183 post-transplant was 78.5 ± 3.8 in SRL group versus 88.4 ± 3.4 in CsA group ($P < 0.0001$). Hemoglobin concentration (g/dl) was not significantly different. Only two patients in SRL group presented decreased transferrin saturation rate. Marked erythrocyte microcytosis without persistent anemia was observed in patients treated with SRL and MMF.

Introduction

Sirolimus (SRL, Rapamycin), a potent macrocyclic lactone immunosuppressant, is well known to have myelotoxic properties [1–5]. One hypothesis to explain the myelosuppressive effects is that the drug inhibits signal transduction via the glycoprotein 130 β-chain shared by variety of cytokine receptors, including interleukin-11, granulocyte colony stimulation factor, and erythropoetin, which stimulate the production of platelets, leukocytes, and erythrocytes respectively. It is also supposed that SRL potentiates platelet destruction via augmented platelet aggregation [4]. Mycophenolate mofetil (MMF), a morpholinoethylester of mycophenolic acid, is also very well known for its myelotoxicity, but the mechanism is still not well understood [1,6,7].

So far, several studies with SRL or MMF have reported anemia as adverse effect [2,8–10], including one case of erythroid aplasia caused by MMF [11]. Morphological characteristics of anemia in SRL treated patients have been first reported by Cahill *et al.* [12]. They have observed a decline in mean corpuscular volume (MCV) in lung transplant recipients treated with SRL. In our center, marked erythrocyte microcytosis was also observed in patients treated with SRL and MMF. Therefore, this observation has been evaluated in a subanalysis of a prospective, open label, single center study comparing SRL and CsA as a primary immunosuppressant.

Patients and methods

Study design

The occurrence of a marked erythrocyte microcytosis within 6 months post-transplant was analyzed as part of a prospective, open label, single center study comparing SRL + MMF + prednisone, with CsA + MMF + prednisone. The study protocol was reviewed and approved by the ethics committee of the University Hospital of Basel prior to start. Normal risk *de novo* kidney recipients (panel reactive antibody <25%, negative T- and B-cell crossmatch, no previous graft loss within 3 years post-transplant) were included in the study and

randomized either to SRL or to CsA. All the patients were informed about and gave consent for the study. Recipients from both cadaveric donors and living donors were included. All patients were monitored for graft function and adverse effects for 6 months. Complete blood count including erythrocyte indices (MCV; mean corpuscular hemoglobin, MCH), and blood chemistry were measured regularly at each visit, and iron storage was evaluated by serum ferritin concentration, transferrin saturation rate, and percentage of hypochromic red blood cells (%HYPO) in case of erythrocyte microcytosis.

Immunosuppressive regimens and target blood concentration

Sirolimus was administered at a daily dose of 30 mg for 3 days (including preoperative administration on day −1), followed by 16 mg/day for the next 2 days, and then at an adjusted dose according to trough blood concentration. Target trough concentration was 10–20 ng/ml for 3 months post-transplant and then 8–15 ng/ml. SRL was dispersed either in a tablet formulation or in a nonaqueous solution.

The initial dose of CsA was 300 mg twice daily for 3 days (including one preoperative administration on day −1) and then adjusted according to blood trough concentration. Target trough concentration was 250–350 ng/ml for 3 months and then 150–250 ng/ml. The micro-emulsion formulation of CsA (Neoral®, Novartis Pharmaceuticals, Basel, Switzerland) was administered to all patients.

Additional immunosuppressants consisted of MMF and prednisone. The initial dose of MMF was 1000 mg twice daily, the target trough concentration above 2 μg/ml, and the dose was modified according to trough level. All the patients had intravenous methylprednisolone for 3 days, and prednisone was started at a dose of 0.5 mg/kg once daily from day 3 post-transplant. Prednisone was tapered to 5 mg/day, and continued till 6 months. SRL, CsA and MMF trough concentrations were measured at each visit.

Concomitant medication

All the patients received calcium and vitamin D substitution, and pneumocystis carinii prophylaxis with trimethoprim–sulfamethoxazole for 6 months. Anti-hypertensive therapy consisted mostly of an ACE inhibitor or an angiotensin receptor blocker.

Definition of erythrocyte microcytosis, anemia and iron deficiency

Erythrocyte microcytosis was defined as MCV below 80 femtoliters (fl) [13]. Anemia was defined as hemoglobin concentration <13 g/dl in men and 12 g/dl in women [14]. For the screening of iron deficiency, serum ferritin concentration, transferrin saturation rate and percentage of hypochromic erythrocytes (%HYPO) were measured. Serum ferritin <10 ng/ml or transferrin saturation rate <15% was indicator for iron deficiency [15]. The normal range of %HYPO was under 5% [16].

Analysis of data

Patients on study drug were analyzed. For the statistical analysis, ANOVA with Fisher's protected least significant difference and contingency table analysis with chi-square test were used.

Results

Pretransplant data

Between January 2001 and January 2003, 59 normal risk *de novo* kidney recipients were included in the study and have finished 6-month post-transplant follow-up. Thirty patients were randomized to SRL, and 29 patients to CsA. Thirty-one patients received allografts from cadaveric donors, 16 patients from living related donors, and 12 patients from living unrelated donors. Patient characteristics and pretransplant laboratory data are shown in Table 1. The patients in both treatment groups displayed similar demographic features. Mean pretransplant hemoglobin concentration and MCV were in normal range and did not show any significant differences. No patients in both groups were known to have any hemoglobinopathies (thalassemia, sickle cell disease, etc.).

Post-transplant data

Among 30 patients who were included in SRL group, four patients were withdrawn within 3 weeks post-transplant because of sudden cardiac death, intolerability of SRL, severe hyperlipidemia, and recurrent vascular rejection, respectively. The remaining 26 patients received SRL and MMF for at least 2 months. Afterwards, 10 more patients were withdrawn in the course of follow up till 6 months post-transplant because of recurrent rejection ($n = 6$), severe hyperlipidemia ($n = 1$), or severe musculoskeletal pain ($n = 3$). In the CsA group, three of 29 patients were withdrawn within 3 weeks post-transplant because of severe vascular rejection ($n = 2$), suspected neurotoxicity ($n = 1$), and the remaining 26 patients received CsA and MMF for at least 2 months. Afterwards, five more patients were withdrawn in the course of follow up till 6 months post-transplant due to recurrent rejection ($n = 1$),

Feature	SRL + MMF + Pred ($n = 30$)	CsA + MMF + Pred ($n = 29$)
Recipient age [mean (range)]	49 (19–69)	51 (20–72)
Recipient gender [male(%)/female]	21 (70)/9	19 (65)/10
Donor age [mean (range)]	50 (20–73)	52 (26–74)
Donor gender [male(%)/female]	12 (67)/18	10 (53)/19
Organ donation [n(%)]		
Cadaveric	16 (53)	15 (52)
Living related/living unrelated	8 (27)/6 (20)	8 (27)/6 (21)
Pretransplant hemoglobin concentration (g/dl) [mean ± SD (range)]	12.7 ± 2.1 (7.7–17.6)	12.4 ± 1.8 (8.6–16.4)
Pretransplant MCV of RBC (fl) [mean ± SD (range)]	90.8 ± 5.1 (78.7–101.4)	91.3 ± 4.6 (80.2–98.8)
Pretransplant MCH (pg) [mean ± SD (range)]	31.2 ± 2.0 (26.8–35.3)	31.1 ± 1.9 (26.2–35.3)

Table 1. Demographic characteristics and pretransplant laboratory values of both groups. None of the parameters was significantly different in both groups.

biopsy-proven CsA nephrotoxicity ($n = 3$), or sudden cardiac death ($n = 1$).

One patient in SRL group had to be switched from MMF to prograf on day 90 post-transplant because of severe leukopenia after anti-lymphocyte globulins (ATG) therapy. All the other patients in both groups had MMF during the whole follow-up period.

Six patients of 26 in SRL group and 10 patients of 26 in CsA group received anti-rejection therapy with ATG because of biopsy proven severe interstitial or vascular rejection, and each one patient in SRL and CsA OKT-3 (*P*: NS). In addition, five patients of 26 in SRL group, and 11 patients of 26 in CsA group received ganciclovir as targeted prophylaxis, or therapy for cytomegalovirus disease.

White blood cell count, platelet count and graft function were not significantly different in both groups during the whole follow-up period (Table 2).

The evolution of microcytosis in both groups is shown in Table 2, and Fig. 1. Twenty-one of 26 patients (81%) in SRL group showed either a marked microcytosis (MCV <79 fl, 16 patients), or a tendency to microcytosis (MCV <85 fl, five patients). Among 10 patients in SRL group, who were withdrawn in the course of follow up till 6 months, five patients showed erythrocyte microcytosis before switch from SRL (mean MCV 80.1 fl, range 78.7–81.5). MCV became higher than 85 fl (mean MCV 86.0 fl, range 83.9–88.4; $P = 0.0002$) within 10 days to 3.5 months (median 31 days) after discontinuation of SRL (see Fig. 2). The remaining five patients showed erythrocyte microcytosis neither before nor after switch.

Among 26 patients in CsA group, five patients (19%) experienced at least one episode of erythrocyte microcytosis. In the course of follow up, CsA was switched to SRL in three patients because of biopsy-proven CsA nephrotoxicity, and all of them developed erythrocyte microcytosis [mean MCV (fl) 92.0 ± 0.6 before switch; 78.6 ± 2.1 in 3 months after switch; $P = 0.0005$, see Fig. 2].

The hemoglobin concentration was not significantly different between two groups except on day 91 (see

Table 2. Mean post-transplant laboratory values of both groups (*$P < 0.01$).

	Crea (mmol/l)	Hb (g/dl)	MCV (fl)	MCH (pg)	WBC ($\times 10^9$/l)	Platelet ($\times 10^9$/l)
Day 7						
SRL ($n = 29$)	247 ± 250	9.6 ± 1.5	91.7 ± 4.8	31.1 ± 1.6	**6.4 ± 2.8***	**171 ± 59***
CsA ($n = 29$)	267 ± 220	9.7 ± 1.5	91.4 ± 4.2	31.0 ± 2.0	10.4 ± 2.8	248 ± 67
Day 28						
SRL ($n = 26$)	167 ± 104	10.2 ± 1.6	**88.5 ± 3.9***	30.1 ± 1.5	6.6 ± 2.5	206 ± 96
CsA ($n = 26$)	166 ± 62	10.7 ± 1.6	92.0 ± 4.0	30.6 ± 1.5	8.4 ± 2.8	248 ± 77
Day 61						
SRL ($n = 23$)	153 ± 61	10.3 ± 1.1	**85.8 ± 5.1***	**28.6 ± 1.3***	7.2 ± 2.0	195 ± 71
CsA ($n = 25$)	146 ± 68	11.1 ± 1.9	91.1 ± 4.5	30.3 ± 1.7	8.3 ± 3.3	245 ± 74
Day 91						
SRL ($n = 21$)	151 ± 45	**10 ± 1.5***	**82.5 ± 4.4***	**27.6 ± 1.1***	6.4 ± 2.2	214 ± 80
CsA ($n = 23$)	164 ± 61	11.5 ± 2.0	90.6 ± 4.7	30.1 ± 1.9	7.4 ± 3.0	255 ± 79
Day 183						
SRL ($n = 16$)	145 ± 39	11.2 ± 1.0	**78.5 ± 3.8***	**26.2 ± 1.1***	6.1 ± 1.7	244 ± 89
CsA ($n = 21$)	148 ± 37	11.7 ± 1.4	88.4 ± 3.4	29.4 ± 1.6	6.8 ± 2.5	255 ± 68

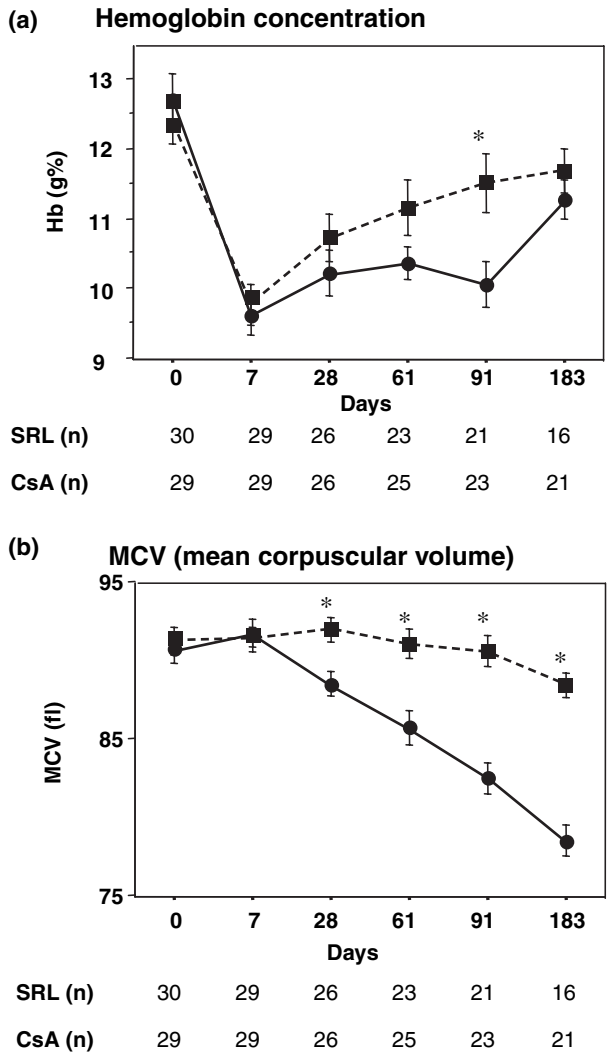

Figure 1 Comparison of hemoglobin concentration (a) and MCV (b) between the SRL-MMF (●) versus CsA-MMF (■) treatment groups over time posttransplant (in days) (*$P < 0.01$).

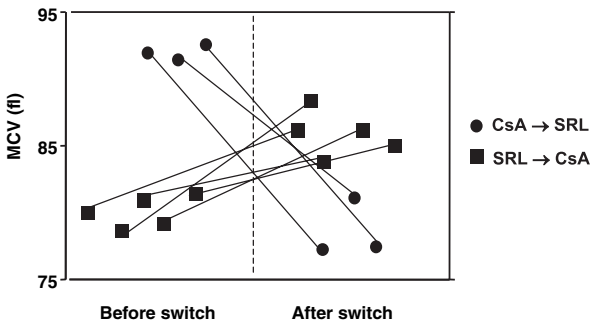

Figure 2 Comparison of MCV (fl) before and after switch from SRL to CsA (■), and from CsA to SRL (●), respectively

Table 2 and Fig. 1a). Compared with hemoglobin, mean MCV of SRL group displayed significant differences to CsA group constantly. Figure 3a,b compares the pretransplant and post-transplant distribution of MCV between two groups. The distribution was similar in both groups prior to the transplantation (3A), while it became significantly different in 6 months post-transplant (3B). MCH was also significantly lower in the SRL group since day 61 (Table 2). Despite low MCV and MCH, most patients in the SRL group showed only mild anemia with hemoglobin concentration between 10 and 12 g/dl.

In all patients showing microcytosis in SRL group, iron storage profile was measured, and 19 of 21 patients showed normal finding (serum ferritin >10 ng/ml, transferrin saturation rate >15%). Two patients showed transferrin saturation rate of 6% and 10% respectively. Regarding %HYPO, only two of 21 patients showed increased %HYPO (>5%) simultaneously with low MCV level.

The trough concentration of SRL during the study follow up was 17.1 ± 7.8 ng/ml on day 7, 20.2 ± 5.8 ng/ml on day 91 and 16.3 ± 3.8 ng/ml on day 183. The trough

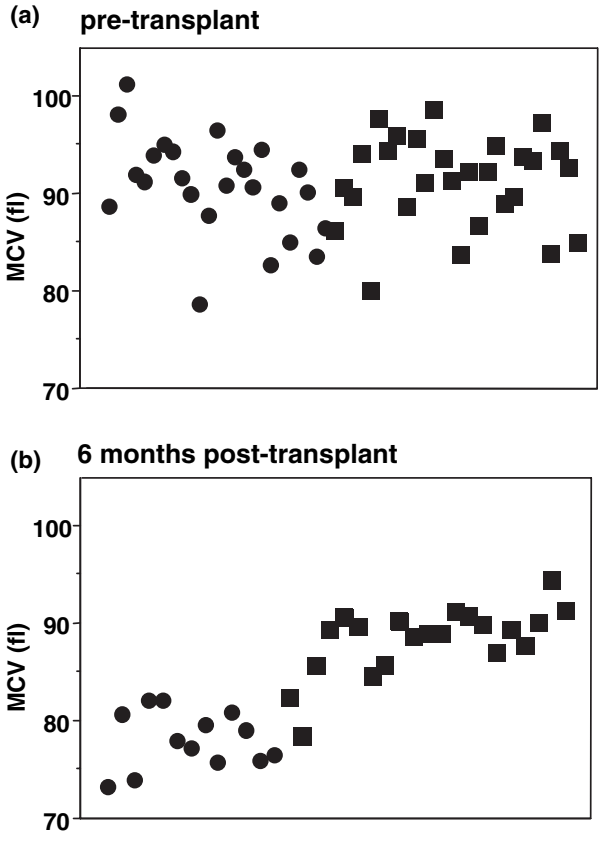

Figure 3 Comparison of MCV distribution between the SRL-MMF (●) versus CsA-MMF (■) treatment groups: (a) pretransplant, (b) 6 months post-transplant.

Table 3. Post-transplant infection. The incidence was not significantly different in both groups.

Infections	SRL ($n = 26$)	CsA ($n = 26$)
CMV infection/disease	4	9
Pneumonia	3	1
Sepsis (catheter-/uro-)	4	3
Invasive aspergillosis	0	1
Enteritis (*C. difficile*, *C. jejuni*)	0	2
Perianal abscess	1	0

concentration of MMF (μg/ml) on day 7 was significantly higher in SRL group (2.9 ± 1.6 in SRL vs. 1.6 ± 0.7 in CsA; $P = 0.003$), but similar on day 91 (3.7 ± 1.4 in SRL vs. 2.9 ± 1.8 in CsA; $P = 0.15$), and on day 183 (4.0 ± 2.2 in SRL vs. 3.1 ± 1.9 in CsA; $P = 0.19$). The dose of MMF (g/day) was significantly higher in CsA on day 91 (1.76 ± 0.77 in SRL vs. 2.39 ± 0.62 in CsA; $P < 0.01$), but not significantly higher on day 7 (2.11 ± 0.46 in SRL vs. 2.38 ± 0.43 in CsA), and on day 183 (1.64 ± 0.69 in SRL vs. 2.15 ± 0.78 in CsA).

Among 21 patients in SRL group, who showed erythrocyte microcytosis, five patients with insufficient renal function (creatinine clearance <40 ml/min) were treated with rHu-Epo. Two patients were treated from day 7 and 33, respectively, three from day 138, 150 and 165 post-transplant. Three of them were treated for 1 month, one for 2 months, and one for 5 months. Among 26 patients in CsA group, three patients were treated with rHu-Epo from day 19, 60, and 150 respectively. Two of them were treated for 2 weeks, and one for 2 months. 73% of patients in SRL group (19/26) and 88% in CsA group (23/26) received either an ACE inhibitor or an angiotensin receptor blocker. In SRL group 15 patients had ACE inhibitor, three patients angiotensin receptor blocker, and one patient both of them. In CsA group 18 patients had ACE inhibitor, and five patients angiotensin receptor blocker (*P*: NS).

The incidence of infections was not significantly different in both groups during the study follow up (Table 3).

Discussion

Several studies with SRL in differently combined regimen have been performed so far [3,8,17–19]. Commonly observed hematologic side effects of SRL and azathioprine (AZA) combination therapy were thrombocytopenia and leukopenia. They occurred in the early post-transplant period [8]. In case of SRL and CsA combination, leukopenia, thrombocytopenia and slower recovery of hemoglobin were reported, which are considered to be strongly associated with SRL trough concentrations [3,19, 20]. Under the combination of SRL and MMF, thrombocytopenia and leukopenia were predominantly observed in the first 3 months post-transplant, also related to high concentration of SRL [17,18,21]. Cahill *et al.* observed a decline in MCV in lung transplant recipients, who were switched from MMF or AZA to SRL in combination with CsA. Patients who had previously received AZA or MMF were equally affected. Iron studies were consistent with anemia of chronic disease. There was no correlation between fall in MCV and rise in serum creatinine [12].

The data of the present study show the occurrence of a marked erythrocyte microcytosis in patients treated with SRL–MMF as compared with patients treated with CsA–MMF. The microcytosis develops within 1 month post-transplant and persists as long as the regimen with SRL–MMF is given. The microcytosis is independent of the extent of anemia, white cell or platelet count. As a possible etiology for microcytosis, iron deficiency could be excluded in most patients by measuring ferritin and transferrin saturation rate. In addition, only two of 21 patients showed increased %HYPO (>5%) simultaneously with low MCV level. Therefore the microcytosis is even more unlikely to be related to iron deficiency.

The insufficient transplant function does also not seem to be the etiology of the microcytosis either, as it usually presents normocytic anemia. Moreover, the renal function improved constantly in the course of time, but microcytosis aggravated inversely. The mean creatinine concentration was similar in both groups, but erythrocyte microcytosis was markedly observed only in SRL group.

The microcytosis cannot be explained by chronic infection, as there was no significant difference in both groups regarding the incidence of infections (Table 3).

The trough concentration of SRL in previous studies was 30 ng/ml for 2 months and 15 ng/ml afterwards [8,17]. In our study, the mean SRL trough concentration was about 20 ng/ml on day 91 and 16 ng/ml on day 183. Although the trough concentration became lower over time post-transplant, erythrocyte microcytosis continued, and therefore trough concentration dependent effect seems unlikely.

The marked microcytosis was predominantly observed under combined therapy with SRL and MMF, but disappeared after discontinuation of SRL, and was not observed, if MMF together with CsA was given (Fig. 2). According to a previous study, SRL increases the trough concentration of MMF and augments myelotoxicity [22]. In our study, MMF trough concentration was similar in both groups except on day 7. The microcytosis, therefore, cannot be explained by higher MMF trough concentrations. We have also evaluated patients in our center, who have SRL in combination with tacrolimus or AZA. Seventeen patients were observed and 12 patients (70%) showed also marked erythrocyte microcytosis.

Therefore primarily SRL, and not the combination therapy with MMF, seems to be responsible for the microcytosis. Nevertheless the presented data do not exclude a combined effect of SRL and MMF on the erythrocyte morphology.

While microcytosis aggravated, anemia resolved spontaneously during the follow up in most patients. Five of 21 patients with insufficient renal function in SRL group received rHu-Epo therapy. Three of 26 patients in CsA group also received rHu-Epo therapy. In the further follow up, 15 patients, who received SRL and MMF combination for 7–24 months (median 12 months), showed mean MCV of 77.5 fl (range 69–83.8), but mean hemoglobin increased further to 12.3 g/dl (range 9.3–14.6). This finding supports that the microcytosis under SRL is not related to iron deficiency. In iron deficiency anemia, moderate to severe anemia should be present before microcytosis appears [23].

In case of iron deficiency anemia, a protein kinase called heme-regulated translational inhibitor phosphorylates the alpha subunit of the translational initiation factor eIF2, and the eIF2alpha protein kinase then downregulates globin synthesis [24]. SRL inhibits selectively the synthesis of ribosomal proteins, and the induction of mRNA for new ribosomal proteins. SRL may inhibit heme synthesis, and thereafter globin synthesis by similar mechanism as in iron deficiency, or may inhibit only globin synthesis under normal heme synthesis. As our patients showed relatively high hemoglobin concentration out of proportion to low MCV, one could guess that the microcytosis may be caused rather by a globin production defect as in globin production disorder, thalassemia [23].

In conclusion, marked erythrocyte microcytosis without significant anemia was shown in patients treated with SRL. This finding is not likely to be associated with SRL trough concentration, and it seems to be reversible after the discontinuation of SRL. Whether SRL alone or the combination with MMF is responsible for the microcytosis remains unclear.

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