Antiproliferative therapy with sirolimus and propranolol for congenital vascular anomalies in newborns (Case reports)

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Abstract. We present a series of four newborns diagnosed with complicated congenital vascular anomalies, with different localization: Congenital lymphatic malformation (CLM) on the left hemithorax extending on the left upper limb; congenital hepatic hemangioma (CHH) with important complications in the first 7 weeks of life; Kaposiform hemangioendothelioma (KHE) of the left lower limb complicated with Kasabach-Merritt phenomenon (KMM) and most probable diffuse capillary malformation with overgrowth (DCMO). All patients were treated with combined antiproliferative therapy with sirolimus and propranolol. The initial dose of sirolimus was 0.45-0.5 mg/m² with doses adjusted according to plasmatic levels. Therapeutic intervals of sirolimus were considered at plasmatic levels of 7-12 ng/ml. Our aim was to use the lowest therapeutic dose in order to avoid possible side effects. Propranolol was initiated in doses of 0.5-1.0 mg/kg/day and was increased up to 3.0 mg/kg/day depending on tolerability. Following two months, every patient showed a marked reduction in the size of the mass, improvement in overall appearance or even calcification in the liver vascular tumor. No patient showed life threatening side effects to the treatment. Hypertriglyceridemia was the only side effect noted in all patients. This is in accordance with several international studies, which try to demonstrate the importance of sirolimus in neonatal vascular malformations in monotherapy or combined with different drugs.

Introduction

Vascular anomalies describe two pathological entities: Congenital vascular malformations (CVMs) and vascular tumors (1). Depending on the presence of arterial flow, these can be described as fast-flow [vascular malformations-arteriovenous malformation, arteriovenous fistula, and complex malformations such as Parkes-Weber syndrome and vascular tumors (hemangiomas or neoplasms such as Kaposiform hemangioendothelioma (KHE), hemangiopericytoma, angiosarcoma)] and low-flow, benign lesions [venous malformations, lymphatic malformations, capillary malformations such as diffuse capillary malformation with overgrowth (DCMO), combined/complex malformations, such as Klippel-Trenaunay syndrome (KTS), Proteus syndrome, CLOVES syndrome, Sturge-Weber syndrome] (2-4).

CVMs result from a failure in angiogenesis during the development of the vascular system; they are present at birth and usually grow with the infant (3). If arterial flow is observed through imaging studies, differential diagnosis includes arterio-venous malformations, arteriovenous fistula or a complex vascular malformation (2).

Capillary malformations are clinically diagnosed and present as port-wine stains and telangiectasia. Most of the times they are isolated anomalies but can be part of a complex vascular malformation (1,3-5).

Venous malformations and complex malformations with a venous component have a variable presentation depending

Abbreviations: CLM, congenital lymphatic malformation; CHH, congenital hepatic hemangioma; KHE, Kaposiform hemangioendothelioma; KMP, Kasabach-Merritt phenomenon; DCMO, diffuse capillary malformation with overgrowth; CVM, congenital vascular malformation; KTS, Klippel Trenaunay Syndrome; PI3K, phosphatidylinositol 3-kinase; AKT, protein kinase B (also PKB); mTOR, mammalian target of rapamycin receptor; HDL, high-density lipoprotein; VEGF, vascular endothelial growth factor

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on their depth and associated complications. Complications associated with venous malformations are venous stasis, thrombosis and localized intravascular coagulopathy (6). Despite being congenital, venous malformations usually manifest later in childhood because they grow with the patient. Upon physical examination, they appear as compressible bluish colored, sponge-like masses (3,6).

Lymphatic malformations appear following aberrant morphogenesis of primordial lymphatic structures and can be classified as microcystic or macrocystic (7-9). Lymphatic malformations or complex vascular malformations with a lymphatic component can lead to disfigurement caused by tissue hypertrophy and skeletal overgrowth; depending on the organs involved, they can lead to chylous effusions with organ compromise. Large lesions can be complicated by fluid loss, hypoproteinemia, bleeding, and infection (10).

Vascular tumors develop from the endothelium; they are fully formed at birth and are divided into congenital hemangiomas that can either be rapidly involuting or non-involuting and more complex tumors such as KHE or tufted angioma (2). Complex vascular neoplasms are infiltrative lesions and can cause Kasabach-Merritt phenomenon (KMP). KMP is a consumptive coagulopathy syndrome consisting of platelet trapping with profound thrombocytopenia, enlargement of the lesion and significant hypofibrinogenemia (10-12).

Currently, there is no established standard of care for the treatment of vascular anomalies. Therapy is guided based on symptoms and association of complications. Management is comprised of surgical excision, sclerotherapy or embolization (2,3,13). Medical management includes steroids, vincristine and interferon with potential significant side effects in infants, especially neurotoxicity (10,14). Propranolol represents the first line of treatment for patients with vascular tumors such as hepatic hemangiomas (14-16).

Ideally, therapies for vascular anomalies would target specific cellular pathways involved in abnormal cellular proliferation and growth.

In the last few years, multiple cases have been reported for the safety of sirolimus as a therapeutic option for various congenital vascular anomalies (9,10,12,17-23). The phosphatidylinositol 3-kinase (PI3K)/protein kinase B (AKT) signaling pathway is pivotal for cellular functions such as growth and survival and has been demonstrated to be involved in normal vascular development and angiogenesis (24,25). Extracellular signals activating the PI3K/AKT pathway transfer signals to mammalian target of rapamycin receptor (mTOR) which, in return, increases expression of vascular endothelial growth factors (VEGF)-A and VEGF-C. VEGF-A and VEGF-C are regulators of both angiogenesis and lymphangiogenesis, promoting protein synthesis, cellular growth and proliferation (9,10,24,25). Over time, inhibitors that target this signaling pathway have been shown to reduce VEGF secretion and angiogenesis, thus making them a suitable option for the treatment of CMVs (24,25). Sirolimus is a direct antagonist of mTOR that acts by blocking the downstream synthesis of the PI3K/AKT/mTOR pathway resulting in antitumoral and antiangiogenic effects by impairing VEGF production (10,26). mTOR receptors have been shown to be overexpressed in vascular tumors, which makes sirolimus suitable as a treatment option for vascular malformations (24). Similar to sirolimus, propranolol, a nonselective β-adrenergic receptor antagonist that causes vasoconstriction and used as first line of treatment for congenital hemangiomas, inhibits angiogenesis and promotes apoptosis (14) by acting on the PI3K/AKT signaling pathway (27). Studies have shown that protein levels and mRNA of both PI3K and AKT were decreased after propranolol treatment, thus suggesting that a reduction in hemangiomas is related to the inhibition of PI3K and AKT (27-29).

Case reports

We analyzed 4 patients admitted to the Neonatal Intensive Care Unit of ‘Marie Curie’ Emergency Clinical Hospital for Children, Bucharest, Romania between August 2019 and September 2020. All patients were diagnosed with CVMs and treated with combined antiproliferative therapy with propranolol and sirolimus. We analyzed the medical records and summarized the date available into a case series. Our aim was to monitor the patient clinical and radiological responses and possible adverse reactions to the therapy and demonstrate the treatment safety in comparison with other cases reported in the literature. All patients were started on oral propranolol at 1 mg/kg/day with doses adjusted up to 3 mg/kg/day according to the tolerance and sirolimus at an initial dose of 0.4 mg/m² with doses increased according to plasmatic levels. Blood tests were drawn every other week and then monthly to check for possible side effects.

All of our patients were females, admitted to our unit between 1 and 21 days of life. One patient was diagnosed prenatally through fetal-magnetic resonance imaging while 3 newborns were diagnosed in our unit. All patients had distinct congenital vascular anomalies: 1 patient was diagnosed with congenital lymphangioma of the left hemithorax and left upper arm; 1 patient with CHH complicated with liver failure; 1 patient with DCMO; and 1 patient with KHE of the left lower limb. Surgical intervention was intended for 2 patients and later postponed because of the high risk of negative outcomes. Treatment was initiated between 4 days of age and 7 weeks.

Response to treatment was noted beginning with the first days of therapy in all patients through changes in the overall aspect and size of the mass, changes in the overlying skin, and improved mobility. Plasmatic levels of sirolimus were monitored at 7 days of treatment and every other week for the duration of stay in our unit. After a stable level was achieved, plasmatic levels were monitored monthly. Plasmatic levels of sirolimus were difficult to maintain in the recommended ranges. All patients had initially a higher plasmatic level that later decreased. Hypertriglyceridemia was the only side effect observed in all four patients. Duration of treatment varied between 27 weeks and 20 months. Three patients still undergo the treatment while for one patient the treatment was stopped after 48 weeks after complete calcification of the liver mass.

Case 1. A female baby was diagnosed prenatally through fetal MRI with lymphatic malformation on the left hemithorax and left upper arm. The newborn was transferred to our unit at 21 h of life, hemodynamically stable, breathing unaided. On inspection, she presented with a giant lesion on the left hemithorax (~30/25 cm) extending to the left upper limb; overlying skin was purplish and there was a central area of telangiectasis (Fig. 1A). There were no differences in color or temperature.
between the two upper limbs; grasping reflex and spontaneous movements were present.

Chest angio-computed tomography (CT) at 4 days of life (Fig. 1B) showed a massive [12/8.5 cm (axial), 13.8 cm (longitudinal)] soft tissue, cystic lesion with fine septal and intralesional hemorrhage. The lesion infiltrated the interscapulo-costal space without damaging the scapulo-humeral articulation. The CT scan revealed only one fine vascular branch inside the mass.

Surgical removal of the lesion was attempted and later postponed following significant hemorrhage after a fine needle aspiration was performed in the operating room. Monotherapy with propranolol was started on admittance but after 4 weeks of treatment there were no visible effects. On the 20th day of life, we began combination therapy with sirolimus (0.4 mg/m2 per dose, twice daily) and propranolol. After the first week of combined therapy, the overall size of the mass shrunk, the overlying skin changed to a natural color and the arm movement improved.

Plasmatic levels of sirolimus were monitored and varied between 2.7‑29 µg/l with doses adjusted between 0.32 and 0.66 mg/m2.

The only side effects noted were a slight tendency to prolonged dyslipidemia with hypertriglyceridemia and decreased levels of high-density lipoprotein (HDL). Massive reduction of the lesion size was noted at 3 months of age (Fig. 1C). Control angio-CT performed at that time (11 weeks of combined therapy) showed a reduction in mass size predominantly through reduction of the cystic component (maximum axial size, 10/5.7 cm) (Fig. 1D). The patient was discharged at home at 3½ months of age, continuing the combined antiproliferative therapy. Currently, she is still undergoing treatment going on 20 months of combined therapy and is on clinical, pharmacological and radiological follow-up.

Case 2. A female baby at clinical examination presented with abdominal distention and a palpable mass in the right hypochondrium. She was diagnosed at the local maternity department with CHH. The diagnosis was based on the angio-CT that showed characteristic findings suggestive of CHH: A well-defined solitary lesion of ~66.5/61.5/66 mm in the central hilar portion of the liver with centripetal enhancement and central sparing (Fig. 2A). Her evolution was complicated by moderate anemia, thrombocytopenia, progressive hepatic cytolysis, cholestatic and inflammatory syndromes. She was transferred to our clinic at 21 days of life.

Upon admittance, the patient was breathing smoothly, had a stable heart rate and was hemodynamically stable; she was moderately jaundiced, had a distended abdomen and a palpable mass of about 7/6 cm was found on clinical examination.

Abdominal ultrasound (US) on admittance showed a central hilar lesion occupying the Vth and VIth liver segments and partially the IVth, VIIth and VIIIth segments; the tumor had well-defined margins, was heterogeneous and had a central inhomogeneous necrotic zone surrounded by a peripheral, well vascularized area (Fig. 2C). Echocardiographic findings were normal.

Upon arrival, she was started on oral propranolol with an initial dose of 1 mg/kg/day which was increased slowly until a maintenance dose of 3 mg/kg/day was reached. Ursodeoxycholic acid was initiated for the cholestatic syndrome at a dose of 10 mg/kg/day. In evolution, the patient exhibited an associated feeding intolerance with frequent postprandial regurgitation and vomiting because of the mass effect of the lesion.

Abdominal US studies showed no visible changes after 4 weeks of treatment. Liver function tests remained elevated over the normal reference ranges and she presented with persistent hypoalbuminemia, for which albumin infusion was
needed. Cholestasis persisted over the course of the treatment with propranolol.

Giving the large size and central localization of the lesion, after a multi-disciplinary hearing composed of the neonatology, pediatric surgery and radiology teams, a decision was made to postpone the surgical treatment, given the high risk for a negative outcome.

At the age of 1 month and 3 weeks, the patient was started on combined antiproliferative therapy with oral sirolimus (0.4 mg/m² per dose, twice daily) and propranolol (3 mg/kg/day). The dose of sirolimus ranged between 0.35 and 0.8 mg/m², adjusted according to plasmatic levels (4.4-16.73 µg/l).

Abdominal US after 1 week of combined antiproliferative treatment showed diminished intratumoral vascularization and new necrotic areas inside the tumoral tissue. After 2 weeks of treatment, a reduction in size was noticeable (63/56/57 mm compared to ~66.5/61.5/66 mm) (Fig. 2D). Side effects noted were persistent dyslipidemia with hypertriglyceridemia and low HDL-cholesterol levels. Control angio-CT scan after 4 weeks of treatment showed moderate reduction of mass size (63/54/51 mm) and multiple intratumoral calcifications (Fig. 2B). The patient was discharged at home at 3 months and 1 week of age on oral propranolol (3.5 mg/kg/day) and sirolimus (~0.55 mg/m²). At present the patient is well at home.

After 30 weeks of treatment, abdominal US showed that the mass shrunk to half of its original size (current size 32/29 mm vs. ~66.5/61.5/66 mm at birth) and is fully calcified.

Combined antiproliferative therapy was stopped after 48 weeks of treatment. Currently she is under clinical and radiological follow-up.

Case 3. A female newborn presented with a giant mass encompassing the left thigh, knee and calf. In evolution, the mass extended upwards to the inguinal region and downwards to the distal 1/3 of the calf and foot; the limb became infiltrated with purplish overlaying skin and tender to touch. She was transferred to our unit at 4 days of age, breathing unaided and hemodynamically stable. Upon inspection, the left lower limb was swollen. A giant mass was noted with ill-defined margins, purplish in color with overlaying petechiae and telangiectasia. The limb was tender to touch and warmer than the right lower limb; the left thigh girth upon arrival was ~25 cm compared to 11 cm around the right thigh, and active movement of the left limb was limited (Fig. 3A). Peripheral pulses were present.

Laboratory tests showed profound thrombocytopenia (PLT 5,000/mm³) and coagulation disturbances requiring administration of FFP and platelet transfusions. Upon arrival, she was started on combined antiproliferative therapy with propranolol (1 mg/kg/day) and sirolimus (starting dose 0.4 mg/m²) in association with methylprednisolone (2 mg/kg/day).

Angio-CT scan performed on admission showed a massive hypervascular, relatively well-defined lesion encompassing the left thigh and knee that receives arterial flow from the inferior segment of the left femoral artery and left popliteal artery with early enhancement of the femoral vein; soft tissue edema of the left labia and thigh was present (Fig. 3B).

Response to treatment was noted starting the first days of therapy. Upon inspection, the foot and calf were slowly normalized in regards to color, swelling of the limb was diminished as well as the petechiae and telangiectasia, margins of the lesion became well defined, encompassing 2/3 distal thigh and 1/3

Figure 2. Case 2. (A and B) Abdominal angio-CT scan showing a solitary hepatic mass with centripetal enhancement and central sparing vs. after 5 weeks of combined antiproliferative therapy which showed a reduction in size with increased intraleisional calcifications. (C and D) Abdominal ultrasound (US) on admittance showing a giant liver lesion with a central necrotic zone surrounded by a peripheral well vascularized area vs. US at 2 weeks of combined therapy which showed increased central necrotic area and intraleisional calcifications. CT, computed tomography.
proximal calf (Fig. 4A and B). Platelet count increased gradually in the first 3 weeks of treatment and normalized after 4 weeks of combined therapy when the PLT count was elevated >200,000/µl. Corticosteroid therapy was tapered over the course of 4 weeks and stopped when the platelets reached a normal value.

After 11 weeks of combined antiproliferative therapy, during which the patient showed good response to therapy, her status was complicated with swelling of the whole left lower limb including the left major labia. The diameter of the thigh increased from 24 to 31 cm in a week. Mobility of the limb was good, overlaying skin color was normal and there was local warmth compared to the contralateral limb. Laboratory tests were normal. Doppler-US of the leg showed no arterial or venous obstruction; control angio-CT showed diffused edema of the leg without changes in tumor size.

Propranolol and sirolimus doses were doubled and methylprednisolon for 4 weeks was reintroduced with no visible effect. At 13 weeks of combined therapy, the patient was started on vincristine at 0.05 mg/m²/week. After 8 rounds of vincristine therapy associated with sirolimus and propranolol, we observed only mild changes in the tumor aspect. Initially, there was a reduction in thigh girth with resolution of labial edema but after 5 rounds of vincristine the thigh girth increased to 32 cm. Because of severe hypertriglyceridemia, we were forced to reduce the dose of sirolimus to 1 mg/m². Vincristine therapy was discontinued after 8 courses.

Throughout the course of the treatment, sirolimus doses ranged between 0.5 and 2.0 mg/m² with plasmatic levels of 5.2-14 µg/l.

The patient is a social case and was transferred to a chronic care facility still undergoing combined therapy, being on week 30 of treatment.

Case 4. A female newborn presented in the delivery room with cyanosis predominantly on the inferior limbs, hypertrophy on the left side of the body with port-wine stains on the inferior limbs and trunk. Upon admission, she was breathing unaided and was hemodynamically stable. Port-wine stains were observed on inspection, predominantly on the left lower limb with extension on the truncal skin, while the left side of the body was hypertrophied apparently through soft tissue overgrowth (Fig. 5A). There was a 2 cm difference in girth between the left and right inferior limbs.

Laboratory tests found elevated D-Dimers with normal fibrinogen and coagulation studies. She was started on sirolimus (0.5 mg/m²) upon admission and was administered combined antiproliferative therapy starting on day of life 10. The initial dose of propranolol was 0.5 mg/kg/day and it was
slowly increased up to 3 mg/kg/day. Whole exome sequencing was performed and came back negative for KTS.

Angio-CT scan performed at 3 weeks of life showed soft tissue asymmetry on the limbs, thorax and abdominal wall and diffusely delimited dense areas of fluid/parafluid consistency in the soft tissue, without any signs of associated vascular anomalies (arterial or venous) (Fig. 5B-D). CT scans incidentally found incomplete transposition of the inferior vena cava to the left of the aorta in the infrarenal segment (suprarenal segment of the IVC at the right of the aorta).

After 7 days of treatment, port-wine stains started to become paler and the diameter of both left limbs decreased mildly. The sirolimus dose was tapered according to the plasmatic levels. The initial dose of 0.4 mg/m² was decreased to 0.3 and then 0.2 mg/m² based on plasmatic levels that ranged from 16.8 to 26 µg/l. The only side effect noted was inconsistent hypertriglyceridemia.

The patient was discharged at home at 7 weeks of life.

Currently, she is on week 27 of combined therapy and is on clinical and pharmacological follow-up.

Discussion

Congenital vascular anomalies are a heterogenous group of pathologies comprised of vascular tumors and CVMs. Reported incidence of CVMs is 0.3-1.5% while vascular tumors such as hemangiomas appear in 2-3% of newborns (1).

We reported a case series of four patients admitted to our NICU Unit between August 2019 and September 2020, diagnosed with distinct vascular anomalies [CLM, CHH, KHE and DCMO] complicated with intralesional bleeding, liver failure or consumptive coagulopathy.

Currently there is no consensus regarding the standard therapy for CVMs, as literature dealing with vascular anomalies consists mainly of case reports. Treatment options are individualized based on the type of malformation, localization, associated symptoms and complications.

Sirolimus formerly known as rapamycin is a mTOR inhibitor with antiproliferative, immunosuppressive and antitumoral effects acting on the mTOR/PI3K/AKT pathway (9). The mTOR/PI3K/AKT pathway is a cell signaling pathway implicated in both angiogenesis and lymphangiogenesis resulting in cellular growth and vascular proliferation (19,24,25,30,31). Sirolimus inhibits aberrant vascular proliferation by blocking the mTOR/PI3K/AKT pathway and decreasing VEGF production (9,26,31).

A recent paper published by Lin et al evaluated the effect of propranolol on infantile hemangiomas and demonstrated regression of the vascular lesions that was related to inhibition of the PI3K/AKT pathway (27).

Cases 1 and 2 diagnosed with CLM of the left hemithorax (case 1) and CHH (case 2) were both initially started on propranolol without a visible effect after 4 weeks of monotherapy.

Propranolol was initiated with starting doses of 0.5-1 mg/kg/day which was increased gradually up to a dose of 3 mg/kg/day for a better tolerance. Sirolimus was started on the lower recommended dose of 0.4-0.5 mg/m² and was adjusted according to plasmatic levels. Our protocol for monitoring plasmatic levels of sirolimus was to draw the first test 7 days...
after the beginning of the treatment and then every other week
for the duration of the patient admission in our unit. After a
steady plasmatic level was reached, tests were drawn monthly.

Recommended plasmatic levels of sirolimus vary between
reports ranging from 7.5-10, 10-15 and
12-20 ng/ml (9,10,19,22,30) while reported doses for
newborns are 0.4-0.8 mg/m² (9,10,17-19,32,33). Our aim was to
use the lowest therapeutic dose of sirolimus to avoid potential
side effects associated with the drug.

Despite suboptimal plasmatic levels, cases 1, 2 and 3
showed impressive results starting in the first days of therapy.
On the other hand, case 4 diagnosed with DCMO had higher
than recommended plasmatic levels despite receiving lower
doses of sirolimus (maximum plasmatic level of 26 ng/ml
at a dose of 0.2 mg/m²). We could not explain this finding
compared with the other patients in our report.

Studies on adult renal transplant patients on immunosuppres
tive therapy with sirolimus showed variations of plasmatic
concentrations of sirolimus among patients receiving the same
dose and an intrapatient variability in blood concentration.
These variations are dependent on varying contents of enterocyte
P-glycoprotein and CYP3A4 between individuals; both
enterocyte P-glycoprotein and CYP3A4 being implicated in
the metabolism of sirolimus (29).

Case 3 diagnosed with KHE of the left lower limb compli
cated with KMP required associative therapy with propranolol,
sirolimus and methylprednisolone for 4 weeks until platelet
levels reached a normal value.

Currently there is no consensus regarding first line of treat
ment for patients diagnosed with KHE with or without KMP.
Medical therapy for patients with KHE and KMP includes
corticosteroids, vincristine, propranolol, interferon and in
recent years sirolimus (30). Regimens reported in the literature
as first line therapies for patients with KHE are corticosteroid
plus vincristine or corticosteroid plus sirolimus (11,12,30,34).

Despite showing good initial response to combined antiproliferative therapy with sirolimus and propranolol, the patient
developed lymphedema of the ipsilateral limb after 12 weeks
of therapy. Giving the fast increase in thigh girth (8 cm
in 1 week) we decided to increase the dose of sirolimus to
2 mg/m² and reintroduced methylprednisolone. We based our
decision on a reported case by Chinello et al, where sirolimus
was administered in doses up to 3 mg/m² to maintain plasmatic
levels between 7.5-10 ng/ml (30).

Vincristine was introduced during the 13th week of
combined antiproliferative therapy. After 8 rounds of vincris
tine, the patient showed diminished leg swelling (thigh girth
decreased by 2 cm), subcutaneous tissue became softer, less
indurated and mobility of the limb improved.

A retrospective multcenter analysis conducted in 2018 by
Ji et al concluded that lymphedema is a frequent sequela found
in patients diagnosed with KHE and that associated KMP or
sirolimus treatment has no prediction in its development (35).
KHE can predispose the patient secondary lymphedema
through infiltration of the tumor in the lymphatic vessels or
to primary lymphedema because of aberrant formation of the
lymphatic vasculature with insufficient vessels (35-36). Giving
the reported evolution of patients with KHE with long term
follow-up it is difficult to conclude whether vincristine or
sirolimus may have any effect on lymphedema.

Response to treatment for case 4 was noted during the
first days of therapy by changes in the color of the capillary
lesions which became paler. Over time, no major changes were
observed on the overall size of the limbs.

Prior to starting the treatment, all patients had blood tests
drawn to check for baseline levels of complete blood count,
acute inflammatory markers, liver and renal function tests,
lipid profile and plasmatic electrolytes based on the most
common adverse effects of sirolimus.

Adverse effects observed in our patients were concor
dant with reported cases in the literature (9,10,14,17,19-22).
Sirolimus is a new immunosupressant drug originally
approved for use in kidney transplant recipients, but no patient
showed signs of infection/sepsis or hematologic changes
throughout the course of treatment. Long-term administration
at these dosages seems to be safe. All patients showed
increased triglycerides. Cases 1 and 2 showed persistent
hypertriglyceridemia that resolved after ~10 weeks of therapy
while cases 3 and 4 showed inconsistent high triglyceride
levels. Studies conducted on adult patients demonstrated
that sirolimus increases liver synthesis of triglycerides
by increasing lipase activity in the adipose tissue and/or
decreasing lipoprotein lipase activity (37).

Before initiation of treatment, case 2 with CHH had elevated
liver enzymes that remained over the normal reference range
over the course of the first 20 weeks of therapy but we could
not distinguish if this was an adverse effect of sirolimus or if
the liver enzymes were elevated because of tissue destruction
by the liver mass.

In conclusion, combined therapy with propranolol and
sirolimus is a safe therapeutic choice for patients with congeni
tal vascular anomalies with good outcomes and without life
threatening adverse effects. More studies must be conducted
to confirm our findings.

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Availability of data and materials

More information concerning the 4 cases can be obtained
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Authors' contributions

CC conceived the presented idea and the study design, and
provided final approval of the version to be published. AMB
investigated the aspects of the case series. CM developed the
theory and contributed to the computations of the data. SM
and AIB collected the data of the patients for this study and
verified the analytical methods. DS and VM helped supervise
the project in view of the data collected. DAI and RIS inves
tigated the aspects and supervised the findings of this work.
All authors have read and approved the final manuscript for
publication.
Ethics approval and consent to participate

The study was approved by the Ethics Committee of ‘Marie S. Curie’ Emergency Clinical Hospital for Children. Patients who participated in this study had complete clinical data. Written informed consent to participate was signed by the patient’s guardian/next of kin.

Patient consent for publication

Written informed consent was obtained from the minors’ guardian/next of kin for publication of any identifiable images or data included in this article. The corresponding author is in possession of these documents.

Competing interests

The authors declare that they have no competing interests.

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