

## Research

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**Protein-losing enteropathy in patients with Fontan circulation: is it triggered by infection?**Dominik Lenz<sup>1</sup>, Jörg Hambsch<sup>2</sup>, Peter Schneider<sup>3</sup>, Hans-Jürgen Häusler<sup>2</sup>, Ursula Sauer<sup>4</sup>, John Hess<sup>5</sup> and Attila Tárnok<sup>6</sup><sup>1</sup>Research Assistant, Research Laboratory, Department of Paediatric Cardiology, Cardiac Centre Leipzig, University Hospital, Leipzig, Germany<sup>2</sup>Assistant Medical Director, Department of Paediatric Cardiology, Cardiac Centre Leipzig, University Hospital, Leipzig, Germany<sup>3</sup>Director, Department of Paediatric Cardiology, Cardiac Centre Leipzig, University Hospital, Leipzig, Germany<sup>4</sup>Assistant Medical Director, Department of Paediatric Cardiology, German Cardiac Centre, Munich, Germany<sup>5</sup>Director, Department of Paediatric Cardiology, German Cardiac Centre, Munich, Germany<sup>6</sup>Director, Research Laboratory, Department of Paediatric Cardiology, Cardiac Centre Leipzig, University Hospital, Leipzig, GermanyCorrespondence: Attila Tárnok, [tarnok@medizin.uni-leipzig.de](mailto:tarnok@medizin.uni-leipzig.de)

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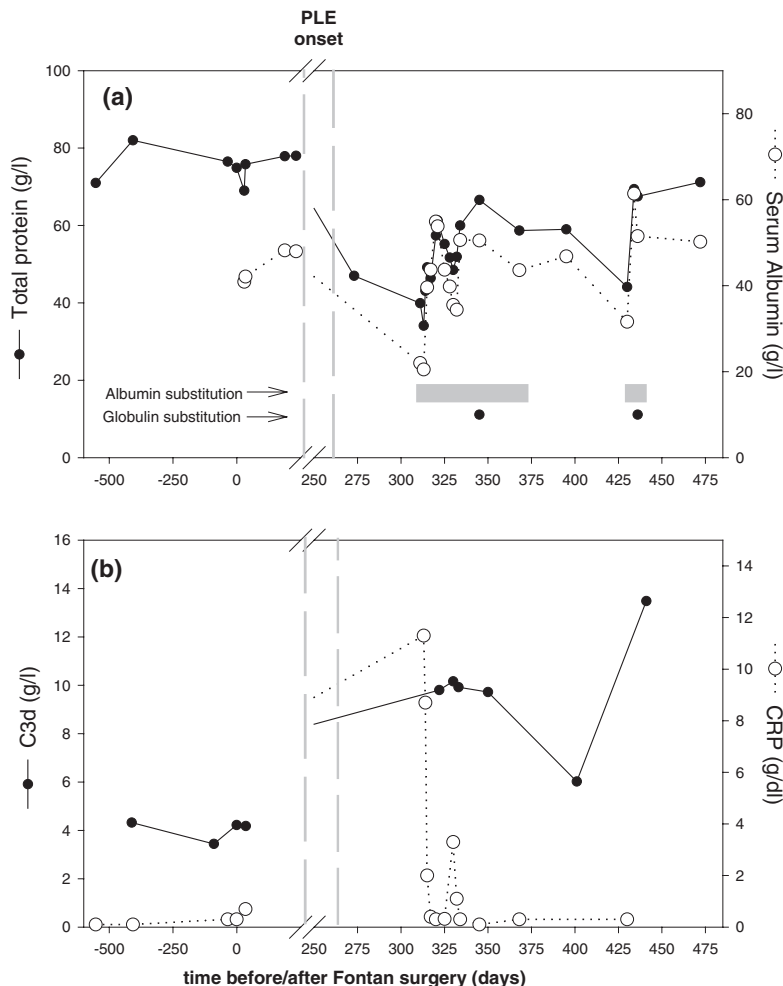
**Abstract****Introduction** Protein-losing enteropathy (PLE) is a recognised complication of the Fontan circulation. Its pathogenesis is not fully understood, however, and it is unclear why its onset occurs months or even years after Fontan surgery.**Patients** We report a 4.5-year-old girl with Fontan circulation who developed PLE almost 1 year after surgery. At the time of onset the patient had rotavirus enteritis and streptococcal tonsillitis. We have reviewed the records of seven other patients with longstanding PLE. In six of these patients we identified infections at the onset of symptoms. None of our patients had evidence of opportunistic infection.**Discussion and conclusion** The immune system of patients with PLE is compromised, but reports on recurrent opportunistic infections are rare. The present observations suggest that infection and inflammation may be associated with the onset of PLE. The mechanism of how infection may trigger PLE warrants further investigation.**Keywords** Fontan type surgery, immunodeficiency, infection, inflammation**Introduction**

The Fontan procedure was developed as a means for separating the systemic and pulmonary circulation in patients with tricuspid atresia, and was then applied to other patients with a functionally single ventricle [1]. Venous hypertension is a general feature of this circulation. A late complication develops in some patients, consisting of a substantial decrease of serum protein and  $\gamma$ -globulin, an increase in stool  $\alpha_1$ -anti-

trypsin level, and a substantial loss of circulating lymphocytes [2–5]. These features are typical of protein-losing enteropathy (PLE), and are associated with significant mortality [4,6–8].

In the present report, we describe our observation of enteric infection and inflammatory response at the onset of PLE in a child with Fontan circulation. We have also reviewed the findings in seven other patients with longstanding PLE.

Figure 1



Time course of selected laboratory data. (a) Time course of the serum protein (solid line, filled circles; left scale) and the serum albumin level (dotted lines, white circles; right scale), and (b) of the complement fragment C3d level (solid line, filled circles; left scale) and the C-reactive protein (CRP) level (dotted lines, white circles; right scale) before, during and after the onset of protein-losing enteropathy (PLE). The vertical hatched line shows the time range of PLE onset. Data are plotted versus time before/after the Fontan procedure.

## Patients and methods

The study was approved by the ethical committees of the University of Leipzig (case 1) and Munich (cases 2–8). Written informed consent was obtained from the parents of the patients. Since 1995, in Leipzig, we have formally followed up children who have undergone the Fontan operation. One child (case 1) out of 30 has developed PLE. We studied seven other patients with PLE, who represented all post-Fontan PLE survivors of the German Heart Centre Munich.

In each case, blood was taken to examine the haemoglobin, the differential blood count, the haematocrit, and the serum protein and globulin subset concentrations. IL-8 and anti-Epstein–Barr virus (EBV) IgG concentrations were quantified by ELISA (R&D Systems, Wiesbaden, Germany and IBL GmbH, Hamburg, Germany, respectively). The concentration

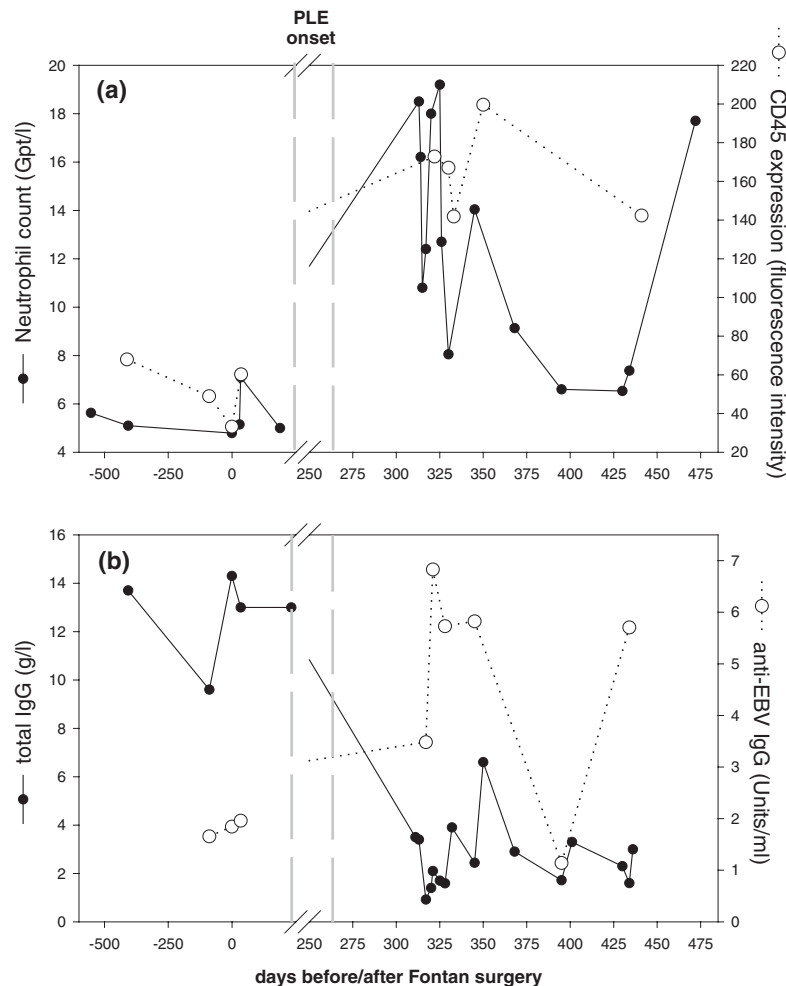
of the complement fragment C3d was determined by radio-immunodiffusion (The Binding Site, Heidelberg, Germany). Surface antigen expression on neutrophils was determined by flow cytometry from EDTA-anticoagulated blood [9] with the antibodies anti-CD45, anti-CD11a and anti-CD11b (all antibodies from BD-Biosciences, Heidelberg, Germany).

## Results

### Case 1

A girl with situs inversus, a double outlet right ventricle, pulmonary atresia and D-transposition of the great arteries who had previously undergone cardiac surgery at the age of 3 months (Blalock–Taussig shunt) and at the age of 3 years (bidirectional Glenn anastomosis) underwent a Fontan-type operation (total cavopulmonary connection, with intra-atrial tunnel) at the age of 4.5 years. She was discharged 14 days

Figure 2



Time course of selected immunological data. **(a)** Time course of the blood neutrophil count (solid black line, filled circles; left scale) and the CD45 expression on neutrophils (determined by flow cytometry and expressed as mean fluorescence intensity; dotted lines, white circles; right scale), and **(b)** the IgG level (solid black line, black circles; left scale) and the anti-Epstein-Barr virus (EBV)-IgG level (dotted lines, white circles; right scale) before, during and after the onset of protein-losing enteropathy (PLE). For further details, see the last two sentences of the legend to Fig. 1.

after surgery, prescribed digoxin, spironolactone, frusemide, acetylsalicylic acid and alprazolam. Six months later the patient's serum protein and albumin levels were within normal limits (Fig. 1a). The laboratory findings are summarised in Table 1 (time range A, before PLE onset).

Eight months later, the patient was admitted to hospital with rotavirus and streptococcal tonsillitis. She subsequently developed oedema, and low serum protein (Fig. 1a) and  $\gamma$ -globulin concentrations. The patient also had an elevated  $\alpha_1$ -antitrypsin level in the stool. The leukocyte count was normal but the neutrophils were left-shifted. There was no proteinuria. The laboratory findings at this time are summarised in Table 1 (time range B). The patient recovered and was discharged after 4 weeks.

Three weeks later (i.e. ~10 months after the total cavopulmonary connection procedure), the patient was referred to our Cardiac Centre because she had suspected infection, again accompanied by oedema and markedly reduced exercise tolerance. On admission the patient was febrile and had oedema of the legs, arms and face. The protein level was very low, and the C-reactive protein concentration (normal value <1 mg/l) (Fig. 1b) and the neutrophil count (normal range, see Table 1) were increased. Serum albumin (Fig. 1a),  $\gamma$ -globulin and IgG concentration (Fig. 2b) was decreased. Although the serum anti-EBV IgG level (Fig. 2b) was increased twofold to threefold, the IgA and IgM concentration was normal. The lymphocyte count was decreased (Table 1). The serum concentration of IL-8 was increased (52.5 pg/ml; normal range, <5 pg/ml). Finally, complement activation (Fig. 1b), and CD45 (Fig. 2a),

**Table 1**

**Laboratory values before (A), at onset (B) and during manifest (C) protein-losing enteropathy (PLE)**

	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6	Case 7	Case 8	Normal range
Age at Fontan surgery (years)	4.5	5.0	6.5	4.0	6.0	6.5	5.0	5.5	
Time of PLE onset after surgery (days)	260	2790	90	2070	720	1530	2160	660	
PLE-associated infection	Rotavirus, streptococcus	<i>Staphylococcus aureus</i>	No Infection	Candidosis	Influenza	Viral pneumonia	Local infection	Viral, fever	
Sampling	Before PLE (A)	A/B/C	A/B/C	A/B/C	A/B/C	A/B/C	A/B/C	A/B/C	
	Onset of PLE (B)	A/B/C	A/B/C	A/B/C	A/B/C	A/B/C	A/B/C	A/B/C	
Total protein	76.5	64/43/55	67/37/37	71/49/42	80/43/66	-/50/39	65/42/66	73/42/36	60-80
Mean (g/l)	46.2								
Range	34-49	44.1-71.2							
Albumin	45.1	-/28/32	49/24/23	42/30/22	46/23/42	-/29/18	44/-/38	-/-/21	35-52
Mean (g/l)	29.3								
Range	20.8-39.0	35.5-50.6							
$\alpha_1$ -globulin	2.9	-/5.6/4.7	3.1/5.1/6.3	2.2/6/8	2.7/7.9/3.9	-/7.5/10.3	3.2/3.2/4.6	-/-/6.1	1.3-3.9
Mean (g/l)	2.5								
Range	2.7-3.0	2.8-3.9							
$\alpha_2$ -globulin	6.2	-/13.9/12.5	7.8/15.3/16	7.7/13.5/19.1	7.4/16.6/8.4	-/14.6/16.5	7.7/-/12.1	-/-/13	5.4-9.3
Mean (g/l)	6.7								
Range	6.0-6.4	5.9-6.9							
$\beta$ -globulin	7.2	-/9.4/16.5	5.8/15.2/11	7.6/10.9/14.7	9/12.1/11.2	-/14.6/16.5	8/8/13.9	-/-/11.8	5.9-11.4
Mean (g/l)	5.2								
Range	7-7.3	4.5-6.0							
$\gamma$ -globulin	13.2	-/5.9/7.6	8.9/2.1/5.9	9.6/8.6/6.5	22.5/9.9/13.5	-/5.4/8.3	14.3/-/12.2	-/7.9/11.7	5.8-15.2
Mean (g/l)	3.5								
Range	13-13.3	2.7-2.9							
$\alpha_1$ -antitrypsin stool	-	-/-/4.4	-/-/27.7	-/-/14.1	-/0.6/-	-/-/45.7	-/54.3/13.0	-/-/9.4	<1
Mean (mg/g)	+								
Range	-	20.2							
		4.8-34							
$\alpha_1$ -antitrypsin serum	180	-/-/260	-/-/160	-	-/332/-	-	-	-/-/183	90-200
Mean (mg/dl)	-								
Range	176-187	-							

Continued

**Table 1****Continued**

	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6	Case 7	Case 8	Normal range
IgG									
Mean (g/l)	12.7	2.9	-/-/2.4	-/-/1.25	-	-	-	-	5.5-15
Range	9.6-13.7	1.4-6.6							
IgM									
Mean (g/l)	1	0.5	-/-/0.6	-/-/0.51	-	-	-	-	0.4-2.1
Range	-	0.2-0.6							
IgA									
Mean (g/l)	1.7	0.4	-/-/32	-	-	-	-	-	0.3-2.3
Range	-	0.2-0.6							
Leukocyte count									
Mean (Gpt/l)	11	17.3	7.8/7.3/7.7	14.3/6.6/6.1	6.3/7.7/6.3	8/5.3/7.5	8.9/9.3/5.7	6.1/11.3/5.6	3.8-13.5
Range	9.5-12.9	8.7-22.1							
Lymphocyte count									
Mean (Gpt/l)	4.3	2.5	2.6/-/0.7	2.8/1.6/0.6	1.6/1.3/1.3	-/-/0.7	3.5/0.9/1.0	2.6/-/0.4	1.5-4.0
Range	3.1-5.8	1.7-4.3							

Mean and respective data ranges. +, value above normal range, without quantitative data in the records; -, no data available. The date of PLE onset in cases 2-8 was not exactly recorded and is estimated.

CD11a (LFA-1; data not shown) and CD11b (Mac-1; data not shown) expression on neutrophils were elevated.

The patient was at this time treated with protein (human albumin, 4 × 50 ml 20%/day), a single immunoglobulin infusion (Sandoglobin®), C1-esterase-inhibitor (Berinert®) and prednisolon (3 × 5 mg/day per orally; during the early phase, in addition, 10-20 mg intravenously) for 5 days and with antibiotics (Cefotiam®) for 6 days. The serum albumin level normalised while the protein level was still at the lower end of the normal range and below pre-PLE levels (Fig. 1a). The serum concentration of C-reactive protein was not increased after 5 days of treatment. Endoscopy and gastric and intestinal biopsies showed no signs of lymphangiectasia. Cardiac catheterisation and angiography revealed minimal stenosis of the right pulmonary artery (pressure gradient, 2 mmHg). Systemic venous and right atrial pressure were moderately increased (mean, 11 mmHg).

The patient was followed up for the next 5 months and continued on prednisolone (5 mg/day), alprazolam, diuretics (furosemide and spironolacton), digitoxin and acetylsalicylic acid. The serum protein, albumin, IgG and  $\gamma$ -globulin levels remained below pre-PLE levels while the neutrophil count, CD45 expression on neutrophils, complement activation and anti-EBV-IgG remained increased (Figs 1 and 2). The lymphocyte count decreased and, on average, was below pre-PLE values (Table 1). Follow-up of the patient ended 480 days after surgery without resolution of PLE. The laboratory findings until final discharge are summarised in Table 1 (time range C). After the infections at the onset of PLE, no further infections were diagnosed until the end of the followup.

### Cases 2-8

The records and investigations of the seven other patients with longstanding PLE were reviewed. The age (mean  $\pm$  SD) at Fontan-type surgery was  $5.5 \pm 0.8$  years, and PLE developed  $48 \pm 32$  months after surgery. Six out of the seven patients (i.e. not case 3) had an infection at the time of onset of PLE. Lymphangiectasia was not excluded in any of these cases. The stool  $\alpha_1$ -antitrypsin level was determined only in cases 5 and 7, and was increased at this time. Protein levels were below the normal range at PLE onset;  $\alpha_1$ -globulin,  $\alpha_2$ -globulin and  $\beta$ -globulin were elevated in most patients, whereas  $\gamma$ -globulin was below the normal range only in case 3 (Table 1, time range B). The leukocyte count was normal in all patients. The lymphocyte count was determined in four patients and was below the normal range in three of them. C-reactive protein was analysed only in case 3, and was 47 mg/dl.

Cases 2-4, 6 and 7 had a decreased lymphocyte count, elevated  $\alpha_1$ -globulin,  $\alpha_2$ -globulin and  $\beta$ -globulin in the serum, and elevated  $\alpha_1$ -antitrypsin in the stool during longstanding PLE. The  $\gamma$ -globulin level remained in the normal range. Complement fragment C3d was  $6.9 \pm 2.1$  g/l (normal range

2–4 g/l) and IL-8 was  $11.1 \pm 6.6$  pg/ml, both values were only slightly increased. Case 5 had normal values (C3d, 2.2 g/l; IL-8, 5.3 pg/ml). Case 4 had recurrent infections with rotavirus, but no subsequent infections were reported in the other six patients.

## Discussion

The principal observation in the present report is that, between 230 and 270 days after the total cavopulmonary connection procedure, in a child with congenital heart disease, we found the development of PLE to be associated with (enteric) infection and an acute inflammatory response. Since we failed to identify any reports about the immunological findings in the early phase of PLE, we reviewed seven other patients with longstanding PLE. In all but one of these patients, infection was associated with the onset of PLE after Fontan surgery.

It is possible that the association between infection and PLE might be a symptom of an impaired immune system during the early development of PLE, rather than a causal factor. However, reports of opportunistic infections in patients with PLE are rare [5,10]. This finding is in agreement with our observation that only one of our patients had recurrent infections. In fact, the present data from case 1 indicate that B lymphocytes are still capable of producing specific antibodies (such as anti-EBV IgG).

As with many retrospective studies, the present study does have limitations. It would have been useful to have undertaken cell function assays and more detailed bacteriological and virological laboratory analysis. We cannot exclude the possibility that infection at PLE onset is a mere coincidence, albeit present in the majority (seven of eight patients; 95% confidence interval, 47–99%). Also, although lymphangiectasia was not found it could have been present in other areas and might have been missed on the biopsies, as proposed by others [3].

Finally, is an 'infection hypothesis' plausible for the development of PLE in Fontan patients? We speculate that it is. All patients with a Fontan circulation exhibit an elevated central venous pressure and an elevated resistance to gut perfusion [4,8]. The thyroid stimulating hormone serum level is reduced, for an extended time, after Fontan surgery [11]. Together, these factors could contribute to an impairment of the enteric immune defence system [12]. Increased pressure is pro-inflammatory for the lung and other organs [13], and thyroid stimulating hormone maintains the gastric mucosal immune defence by intraepithelial lymphocytes [14]. A primed and impaired immune system reacts more severely to stress. An impairment of gastric mucosal defence could, in affected individuals, be more susceptible to various stressors. We hypothesise that infection might serve as a trigger for PLE. Indeed, the infections we identified around the time of onset of PLE are not uncommon in children.

## Key messages

- PLE is a serious complication in a subset of patients with Fontan circulation
- It is unclear what triggers the development of PLE and why it can develop so long after Fontan surgery
- PLE may be the common endpoint of multiple pathophysiological pathways. We hypothesise that infection and inflammation are associated with, and may possibly be, an important additional factor for the development of PLE

## Conclusion

Our observation in postoperative patients with Fontan circulation may indicate that infection could be an additional stimulus for the development of PLE. Such a hypothesis could explain why PLE occurs with varying intervals after surgery.

We suggest that further studies, with an extended number of patients, are needed to understand the potential role of infection in the development of PLE.

## Competing interests

None declared.

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