Research

Protein-losing enteropathy in patients with Fontan circulation: is it triggered by infection?

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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 γ-globulin, an increase in stool α₁-antitrypsin 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.

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
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...
after surgery, prescribed digitoxin, 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 γ-globulin concentrations. The patient also had an elevated α1-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), γ-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 | | | | | | | | |
| Staphylococcus aureus | | | | | | | | |
| No Infection | | | | | | | | |
| Candidosis | | | | | | | | |
| Influenza | | | | | | | | |
| Viral pneumonia | | | | | | | | |
| Local infection | | | | | | | | |
| Viral, fever | | | | | | | | |
| Sampling | Before PLE (A) | Onset of PLE (B) | Chronic PLE (C) | A/B/C | A/B/C | A/B/C | A/B/C | A/B/C | A/B/C | A/B/C | A/B/C |
| Total protein | | | | | | | | |
| Mean (g/l) | 76.5 | 46.2 | 55.6 | 64/43/55 | 67/37/37 | 71/49/42 | 80/43/66 | 73/42/36 | 60–80 |
| Range | 71–82 | 34–49 | 44.1–71.2 | | | | | | |
| Albumin | | | | | | | | |
| Mean (g/l) | 45.1 | 29.3 | 46.5 | –/28/32 | 49/24/23 | 42/30/22 | 46/23/42 | –/29/18 | 44/~38 | –/>21 | 35–52 |
| Range | 42.1–48 | 20.8–39.0 | 35.5–50.6 | | | | | | | | |
| α₁-globulin | | | | | | | | |
| Mean (g/l) | 2.9 | 2.5 | 3.4 | –/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 |
| Range | 2.7–3.0 | – | 2.8–3.9 | | | | | | | | |
| α₂-globulin | | | | | | | | |
| Mean (g/l) | 6.2 | 6.7 | 6.4 | –/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 |
| Range | 6.0–6.4 | – | 5.9–6.9 | | | | | | | | |
| β-globulin | | | | | | | | |
| Mean (g/l) | 7.2 | 5.2 | 5.3 | –/9.4/16.5 | 5.8/15.2/11 | 7.8/10.9/14.7 | 9/12.1/11.2 | –/14.6/16.5 | 8/8/13.9 | –/>11.8 | 5.9–11.4 |
| Range | 7–7.3 | – | 4.5–6.0 | | | | | | | | |
| γ-globulin | | | | | | | | |
| Mean (g/l) | 13.2 | 3.5 | 2.8 | –/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 |
| Range | 13–13.3 | – | 2.7–2.9 | | | | | | | | |
| α₁-antitrypsin stool | | | | | | | | |
| Mean (mg/g) | – | + | 20.2 | –/~4.4 | –/~27.7 | –/~14.1 | –/0.6/~ | –/~45.7 | –/~54.3/13.0 | –/>9.4 | <1 |
| Range | – | – | 4.8–34 | | | | | | | | |
| α₁-antitrypsin serum | | | | | | | | |
| Mean (mg/dl) | 180 | – | – | –/~260 | –/~160 | – | –/~332/~ | – | – | –/>183 | 90–200 |
| Range | 176–187 | – | – | | | | | | | | |

Continued
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 immunglobulin 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 prednisolon (5 mg/day), alprazolam, diuretics (furosemide and spironolacton), digitoxin and acetylsalicylic acid. The serum IgG and γ-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).

### Cases 2–8

The records and investigations of the seven other patients with longstanding PLE were reviewed. The age (mean ± SD) at Fontan-type surgery was 5.5 ± 0.8 years, and PLE developed 48 ± 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 α1-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; α1-globulin, α2-globulin and β-globulin were elevated in most patients, whereas γ-globulin was below the normal range at PLE onset in cases 5, 6 and 7. The stool α1-antitrypsin level was below the normal range in all patients. The leukocyte count was normal in all patients, whereas “lymphocytes” was below the normal range at PLE onset in cases 5 and 7, and was increased at this time in cases 6 and 7.

### Table 1

| Case 1 | Case 2 | Case 3 | Case 4 | Case 5 | Case 6 | Case 7 | Case 8 | Normal range |
|-------|--------|--------|--------|--------|--------|--------|--------|--------------|
| IgG (Mean (g/l)) | 12.7 | 3.4 | 2.9 | –/–/2.6 | –/–/2.4 | –/–/1.25 | – | – | – | – | 5.5–15 |
| Range | 9.6–13.7 | – | 1.4–6.6 | – | – | – | – | – | – | – | – |
| IgM (Mean (g/l)) | 1 | 0.4 | 0.5 | –/–/1.2 | –/–/0.6 | –/–/0.51 | – | – | – | – | 0.4–2.1 |
| Range | – | – | 0.2–0.6 | – | – | – | – | – | – | – | – |
| IgA (Mean (g/l)) | 1.7 | 0.8 | 0.4 | –/–/0.7 | –/–/0.32 | – | – | – | – | 0.3–2.3 |
| Range | – | – | 0.2–0.6 | – | – | – | – | – | – | – | – |
| Leukocyte count (Mean (Gpt/l)) | 11 | 19.3 | 17.3 | 9.4/13.2/6.3 | 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 | 6.1/13.3/5.6 | 3.8–13.5 |
| Range | 9.5–12.9 | 12.5–24.7 | 8.7–22.1 | – | – | – | – | – | – | – | – |
| Lymphocyte count (Mean (Gpt/l)) | 4.3 | 3.7 | 2.5 | –/–/0.8/1.0 | 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.
2–4 g/l) and IL-8 was 11.1 ± 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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