On examination, the patient was afebrile with blood pressure 93/65 mmHg, pulse 93 beats/min and oxygen saturation 97% on room air. She had telangiectasias on her face and chest, sclerodactyly, nail-fold capillary drop-out and right third distal phalanx status post-amputation. She had a 3/6 systolic murmur and a loud pulmonic second sound with parasternal heave. Her lungs were clear, and she had no peripheral oedema. She had normo-active bowel sounds without abdominal tenderness, ascites or hepatosplenomegaly.

Laboratory data showed normal complete blood count and comprehensive metabolic panels except creatinine 1.5 mg/dl (pre-renal; baseline 1.0 mg/dl). Negative tests included serum lactate, complements and CRP; blood and stool cultures; ova and parasite; and PCR for CMV and EBV. ECG showed normal sinus rhythm, right axis deviation and right ventricular hypertrophy without ischaemic changes. CT of the abdomen/pelvis showed bowel wall thickening, submucosal oedema and mucosal hyperaemia with diverticulosis in the sigmoid colon. Colonoscopy revealed a deep cratered ulcer at 50 cm from the anal verge with diffusely decreased vascular pattern in the recto-sigmoid colon (Fig. 1A). Biopsy of the ulcer showed focal mucosal erosion and fibrinous change without significant vascular inflammation, consistent with ischaemia (Fig. 1C). A cine abdominal magnetic resonance angiogram evaluating the mesenteric vascular anatomy and postprandial change in vascular flow, showed normal mesenteric arteries but abnormal postprandial flow in the superior mesenteric vessels following a 200 kcal meal [1]. These findings were compatible with small-vessel ischaemia (Fig. 1D). Hypercoagulability work-up, including aPLs, was negative.

Epoprostenol was uptitrated to 63 ng/kg/min and warfarin re-initiated. Within 1 week, the patient reported improvement in abdominal pain. A flexible sigmoidoscopy 1 month later showed resolution of the ulceration (Fig. 1B).

Vascular complications of SSc include digital ulcers/ischaemic loss, renal crisis and PAH [2]. The most common gastrointestinal manifestation of SSc is not vasculopathy but rather dystomyaltity, affecting up to 90% of the patients [3], and thought to be related to smooth muscle atrophy and fibrosis [2]. The resulting functional impairment can manifest as oesophageal dysmotility, delayed gastric emptying, pseudo-obstruction, faecal incontinence and rarely stercoral ulceration secondary to faecal impaction [4, 5]. Gastrointestinal vasculopathy in the form of GAVE identified on endoscopy. Warfarin was stopped and inhaled iloprost switched to i.v. epoprostenol. Three months before her current admission, mPAP had improved to 57 mmHg and CO to 4.3 l/min on epoprostenol 40 ng/kg/min. One month later, the patient developed intermittent, crammary abdominal pain with occasional nausea, vomiting, diarrhoea and a 12-pound weight loss. CT and flexible sigmoidoscopy showed acute diverticulitis. An empiric antibiotic course did not improve the symptoms.
leading to ulceration. She did not have faecal impaction, making stercoral ulceration unlikely.

Vascular injury early in SSc disease leads to endothelial apoptosis and increased circulating endothelial cells [7]. Subsequent endothelial dysfunction manifests as a decrease in vasodilators (endothelial nitric oxide synthase, prostacyclin synthase) and an increase in vasoconstrictors (ET-1) [8]. The imbalance contributes to adventitial fibroblast activation with resultant intimal proliferation, luminal narrowing and tissue hypoxia [9]. Epoprostenol, a prostacyclin analogue, which enhances vasodilation and inhibits platelet aggregation, has been shown to improve haemodynamics in severe SSc-associated PAH, and to prevent and heal digital ulcers [10].

Since patients with SSc are known to have low-circulating prostacyclin levels, we hypothesized that systemic replacement with epoprostenol would improve blood flow to the ischaemic area in the colon, and that warfarin would counteract the fibrinolytic dysregulation underlying SSc vasculopathy. The patient’s abdominal pain improved on these therapies, and the colonic ulcer healed on sigmoidoscopy 1 month later. Unfortunately, the patient died of right ventricular failure from PAH 3 months later.

Rheumatology key message

- Colonic ulceration in SSc may be a manifestation of underlying vasculopathy.

Acknowledgements

L.C. receives funding support from the Scleroderma Research Foundation.

Disclosure statement: The authors have declared no conflicts of interest.

Lily Kao¹, Parvathi Myer², Linda Nguyen², Roham T. Zamanian¹,³,⁴ and Lorinda Chung¹,⁵

¹Division of Immunology and Rheumatology, ²Division of Gastroenterology, ³Division of Pulmonary and Critical Care
Disease and
cicity of extracellular Grp94 [2]. Hence, there is a possibility
viding indirect experimental evidence of the immunogen-
experiments on plasma of type 1 diabetic patients [1], pro-
immune diseases and development of associated vascular
resident chaperone, in the pathogenesis of inflammatory/
immune diseases, such as RA and SLE [3, 4], and that anti-Grp94 antibodies can be taken as an early and sensi-
tive indicator of the development and evolution of these
diseases [4]. In support of this, the paper of Weber et al. [5] should add further evidence to previous experimental
data, showing the increase of anti-Grp94 antibodies in pa-
tients with RA and SLE. Unfortunately, however, the paper
suffers with several methodological errors that heavily
affect validity of the results. One critical aspect relates to
ELISA for measuring auto-antibodies in serum. The
authors made determinations without previously validating
the assay; i.e., they omitted to construct the calibration
curve with both negative and true-positive samples
to establish the lower and higher limits of sensitivity),
and determine intra- and inter-assay variability [6, 7].
Reproducibility of measures should also be tested using
at least two dilutions of each serum sample in duplicate
and the values, read on the linear portion of the curve,
should be expressed as antibody titre (dilution factor of
serum) or antibody concentration (μg/ml). Normalization
by plasma proteins is preferable, since in patients with
immune diseases (and also in normal subjects) protein
concentration [especially that of immunoglobulin G (IgG)]
cannot be assumed that is equal, and averaging antibody
values without this correction might generate big errors.
It is not clear why the authors used western blotting (WB)
to assess specificity of the immune reaction after having
already made the same measure in ELISA. As a rule, WB is
used first to identify both true-positive and negative sam-
ple batches that then serve for constructing the calibration curve
in ELISA. The only difference between the two methods is
the higher sensitivity and reliability of ELISA, which in add-
ton, permits quantification of measurements. Since the
same antigenic proteins are used in both methods (with
the exception of a lower quantity of antigen in ELISA),
the reason why the authors omitted Grp94 in experiments
of WB to detect true-positive reactions (Fig. 2 in their
paper) appears to contradict the principle of the method
and negating validity of results obtained with
Grp94 in ELISA (Table 1 in their paper).
A matter of concern is also the lack of adequate controls
for excluding non-specific reactions of both serum sam-
ple and control human serum albumin (HSA) and/or IgG at approxi-
mately the same concentration present in patients’ sam-
ple. In particular, the authors do not seem to know that
Grp94 can also irreversibly bind IgG at sites other than the
antigen-binding site [8], thus giving rise to significant
false-positive reactions with IgG antibodies (of different
species). We investigated this crucial property of Grp94 in
death and found that non-specific binding of Grp94 to
IgG could only be prevented by thermal denaturation of

Comment on: Antibodies to the endoplasmic reticulum-resident chaperones calnexin, Bip and Grp94 in patients with rheumatoid arthritis and systemic lupus erythematosus

Sini, The role of Grp94, the most represented endothelium-
resident chaperone, in the pathogenesis of inflammatory/
immune diseases and development of associated vascular
complications has recently been demonstrated in experiments on plasma of type 1 diabetic patients [1], pro-
viding indirect experimental evidence of the immunogen-
ecity of extracellular Grp94 [2]. Hence, there is a possibility
that Grp94 might also display antigenic activity in other
immune diseases, such as RA and SLE [3, 4], and that anti-Grp94 antibodies can be taken as an early and sensi-
tive indicator of the development and evolution of these
diseases [4]. In support of this, the paper of Weber et al. [5] should add further evidence to previous experimental
data, showing the increase of anti-Grp94 antibodies in pa-
tients with RA and SLE. Unfortunately, however, the paper
suffers with several methodological errors that heavily
affect validity of the results. One critical aspect relates to
ELISA for measuring auto-antibodies in serum. The
authors made determinations without previously validating
the assay; i.e., they omitted to construct the calibration
curve with both negative and true-positive samples
to establish the lower and higher limits of sensitivity),
and determine intra- and inter-assay variability [6, 7].
Reproducibility of measures should also be tested using
at least two dilutions of each serum sample in duplicate
and the values, read on the linear portion of the curve,
should be expressed as antibody titre (dilution factor of
serum) or antibody concentration (μg/ml). Normalization
by plasma proteins is preferable, since in patients with
immune diseases (and also in normal subjects) protein
concentration [especially that of immunoglobulin G (IgG)]
cannot be assumed that is equal, and averaging antibody
values without this correction might generate big errors.
It is not clear why the authors used western blotting (WB)
to assess specificity of the immune reaction after having
already made the same measure in ELISA. As a rule, WB is
used first to identify both true-positive and negative sam-
ple batches that then serve for constructing the calibration curve
in ELISA. The only difference between the two methods is
the higher sensitivity and reliability of ELISA, which in add-
ton, permits quantification of measurements. Since the
same antigenic proteins are used in both methods (with
the exception of a lower quantity of antigen in ELISA),
the reason why the authors omitted Grp94 in experiments
of WB to detect true-positive reactions (Fig. 2 in their
paper) appears to contradict the principle of the method
and negating validity of results obtained with
Grp94 in ELISA (Table 1 in their paper).
A matter of concern is also the lack of adequate controls
for excluding non-specific reactions of both serum sam-
ple and secondary anti-human IgG antibodies with the
antigen. The authors used only BSA as control in half of
the plate wells, BSA can be taken as the blank for positivity
due to un-blocked sites in the plate, but the real blank is
made with antigen incubated in the absence of primary antibodies (thus, rigorously speaking, with plasma
proteins other than anti-chaperone antibodies; i.e., with
control human serum albumin (HSA) and/or IgG at approxi-
mately the same concentration present in patients’ sam-
ples). In particular, the authors do not seem to know that
Grp94 can also irreversibly bind IgG at sites other than the
antigen-binding site [8], thus giving rise to significant
false-positive reactions with IgG antibodies (of different
species). We investigated this crucial property of Grp94 in
death and found that non-specific binding of Grp94 to
IgG could only be prevented by thermal denaturation of

References

Rheumatology 2011;50:628–629
doi:10.1093/rheumatology/keq393
Advance Access publication 11 December 2010