Acute lung injury after aortic surgery: the relation between lung and leg microvascular permeability to 111-indium-labelled transferrin and circulating mediators

Pieter G H M Rajmakers, A B Johan Groeneveld, Jan A Rauwerda, G J Jaap Teule, C Erik Hack

Abstract

Background – Aortic surgery is a risk factor for acute lung injury and this may relate to ischaemia/reperfusion (I/R) of the lower body and release of inflammatory mediators. The aim of this study was to define the changes in microvascular protein permeability and circulating inflammatory mediators after aortic surgery.

Methods – In 11 consecutive patients who underwent elective aortic surgery microvascular permeability in lung and leg was measured before and after a median of 2.8 hours after completion of surgery. Using 111-indium (In)-labelled transferrin and 99m-technetium (Tc)-labelled red blood cells, yielding a protein leak index (PLI) that is specific for protein permeability. Circulating leucocyte counts and levels of inflammatory mediators were determined.

Results – In the lung the PLI rose from a median of 0.6 (range –0.5 to 2.2) × 10^{-3} min before surgery to 5.4 (–2.3 to 33.5) × 10^{-3} min after surgery, and in the leg from 0.3 (–1.6 to 1.7) × 10^{-3} min to 5.0 (1.0 to 27.8) × 10^{-3} min. The increase in PLI in the lung was related to that in the leg. Levels of activated complement C3a and tumour necrosis factor-α did not change, but levels of interleukin (IL)-6, IL-8 and elastase-α-antitrypsin increased.

Conclusions – Early after aortic surgery microvascular protein permeability increases in the leg and lung. Leg I/R injury may result in neutrophil activation and release of IL-8, which may induce neutrophil sequestration and subsequently increased pulmonary microvascular permeability. These findings may help to explain the occurrence of acute lung injury after I/R in man.

Keywords: aortic surgery, acute lung injury, adult respiratory distress syndrome, increased permeability.

Aortic surgery with temporary clamping of the aorta in patients with vascular disease may result in ischaemia/reperfusion (I/R) of the lower body, microvascular injury, and increased permeability oedema.1–3 Aortic surgery may also impair gas exchange through microvascular changes in the lungs with arterial hypertension and increased permeability. In some patients these changes may contribute to the development of acute lung injury and adult respiratory distress syndrome (ARDS), and may necessitate prolonged mechanical ventilation after surgery.4–7 In a previous study pulmonary microvascular permeability appeared to increase at about three hours after aortic surgery and to return to normal about 24 hours later.8 A non-invasive dual radionuclide technique was used involving intravenously injected 67gallium (Ga)- and 99m-technetium (Tc)-labelled red blood cells.8,9 This technique yields the transvascular transport rate of 67Ga, the so called pulmonary leak index (PLI), but is relatively unspecific since 67Ga does not bind firmly to circulating transferrin.8–9 In contrast, the PLI assessed with transferrin-bound indium (In) isotopes is considered specific for protein permeability.9,10

Animal studies have shown that I/R of the lower body, involving gut,11 skeletal muscle in hind limbs,12–15 or both, increases pulmonary permeability following neutrophil mediated microvascular changes, independently of the rise in pulmonary artery pressure. Indeed, I/R of gut or skeletal muscle may activate neutrophils and elicit (transient) local permeability oedema.11–15 Subsequent sequestration of activated neutrophils in the lungs is believed to cause the pulmonary microvascular changes. The role of inflammatory mediators in local and remote microvascular changes varies from study to study and the contribution of some of them, such as interleukin (IL)-8, a potent neutrophil activator released by hypoxic/regionoxenated cells in vitro and possibly involved in the pathogenesis of ARDS in man, is unclear.14–22 The clinical counterpart of these experimental observations, including increased permeability in the I/R affected tissue, remains largely unknown.12,16

A study was undertaken to determine (1) whether aortic surgery and I/R in man increase leg and lung microvascular protein permeability, (2) how this contributes, together with pressure factors, to the development of lung radiographic and gas exchange changes after surgery, and (3) whether local and remote...
microvascular changes relate to circulating inflammatory mediators. The latter included neutrophil agonists such as activated complement factor C3a, tumour necrosis factor (TNF)-α, IL-6 and IL-8, leucocyte counts, and the neutrophil degranulation product elastase-α1-antitrypsin.46

**Methods**

**PATIENTS**

Eleven consecutive patients were recruited to the study, with three having an abdominal aortic aneurysm, one a thoracic aneurysm, and seven occlusive aortoiliac disease. Their median age was 64 (range 22–73) years and three were women. The study was approved by the hospital ethics committee and all patients gave informed consent.

**THERAPEUTIC PROTOCOL**

Patients underwent general anaesthesia, intubation, and mechanical ventilation with an oxygen/air mixture. In all patients a catheter was inserted into the radial or femoral artery for monitoring arterial blood pressure and for blood sampling. A balloon-tipped pulmonary artery catheter (Pentacath SP55078HS, Spectramed, Bithoven, The Netherlands) was inserted in nine patients for measurement of the pulmonary capillary wedge pressure (PCWP) and mean pulmonary artery pressure (MPAP), allowing assessment of the potential contribution of pulmonary microvascular hydrostatic pressure to the lung PLI and radiographic changes.67 The other two patients received a catheter in the jugular or subclavian vein to monitor central venous pressure (CVP). All patients received therapeutic doses of heparin during the operation, antagonised by protamine after completion of surgery. The aortic clamping time was a median of 60 (range 30–110) minutes. The interval between aortic declamping and completion of surgery was about 30 minutes. Blood losses were replaced by packed cells, albumin, and saline solutions. After surgery all patients were admitted to the surgical intensive care unit.

**STUDY PROTOCOL**

Microvascular protein permeability in the leg and lung was measured on the day before and a median of 2.8 (range 1.4–4.6) hours after completion of aortic surgery (day 0) in the intensive care unit using a previously described dual radionuclide method.687910 In brief, autologous red blood cells were labelled with 99mTc (300 μCi/11 MBq, physical half life six hours). Ten minutes after injection of the red blood cells, transferrin was labelled in vivo following an intravenous injection of 111In chloride (100 μCi/4 MBq, physical half life 2.8 days, Amersham, UK). During the first hour after the injection blood samples were drawn at 3–5 minute intervals (n=10 in total). Starting at the time of the intravenous injection of 111In, radioactivity was recorded in three minute frames for one hour using two mobile probes. One probe was placed over the upper zone of the left lung and the other over the right leg, 8 cm proximal to the upper edge of the patella. The radioactivity windows were centred (20%) around the photoelectric peaks of the radionuclides: 140 keV for 99mTc and 171 and 245 keV for 111In. 99mTc and 111In counts were corrected for background radioactivity, physical half life, spillover of 111In into the 99mTc window, obtained by in vitro measurements, and expressed as counts per three minute frame. Each blood sample was weighed and radioactivity was measured by a well counter and expressed as counts/min/g. Two windows were used: one window centred around the 140 keV peak of 99mTc and one window including the 171 and 245 keV peaks of 111In. For each blood sample a time-matched count rate over the lung and the leg was taken. For leg or lung (organ) a ratio was calculated, according to (111Inleg/99mTcorgan)/(111Inblood/99mTcblood), and plotted against time. The PLI was calculated using linear regression analysis from the slope of the increase in the radioactivity ratio divided by the intercept. The PLI reflects the transvascular transport rate of 111In-labelled transferrin.687910

On the day before surgery venous blood samples were obtained at the end of the radionuclide study for measurement of leucocyte counts and neutrophil fractions (Coulter JS, Coulter Electronics, Luton, UK), and for levels of inflammatory mediators, including activated complement C3a, TNF-α, IL-6, IL-8, and elastase-α1-antitrypsin. The latter blood samples were centrifuged for 15 minutes at 1500 rpm and plasma samples were stored at −70°C until assayed. At day 0 arterial blood was again sampled for measurement of the above variables and also α1 in heparinised syringes, for measurement of oxygen tension (Corning 178/288, Corning Medical and Scientific, Medfield, Massachusetts, USA). On day 0 all patients were mechanically ventilated with positive end expiratory pressure (PEEP) and a chest radiograph was taken. The inspiratory oxygen fraction (FiO2) and PEEP were taken from ventilator settings. The CVP, PCWP, and MPAP were measured (Viggo-Spectramed, Spectramed, Bithoven, The Netherlands; monitor Tramscope, Marquette Electronics, Milwaukee, Wisconsin, USA) at end of expiration with the patients in the supine position, after calibration and zeroing to atmospheric pressure, at the mid chest level. At 07.00 hours on the first postoperative day (day 1) arterial blood was taken for measurement of PaO2 and a chest radiograph was taken; this was also done on day 2 in case the patient was still mechanically ventilated. In the patients who were not on mechanical ventilation who were receiving supplemental oxygen (100%) per Venturi mask on day 1 the FiO2 was estimated as 0.4 with three litres and 0.5 with five litres of oxygen. Changes in pulmonary gas exchange were judged from changes in the oxygenation ratio – that is, the arterial PaO2/FiO2.

The chest radiographs were scored by one of the investigators who was blinded to the study results: 1=mild interstitial oedema, 2=severe interstitial oedema, 3=alveolar oedema.
in 1–2 quadrants, and 4 = alveolar oedema in 3–4 quadrants. After day 2 patients were followed until discharge from the intensive care unit to evaluate development of ARDS, defined according to clinical criteria, and for clinical signs of ischaemic colitis.

Attending physicians, unaware of the study results, were responsible for all patient management decisions.

ASSAYS

Inflammatory mediators were determined as previously described. In brief, C3a was measured by a radioimmunooassay (normal values 1–5 nmol/l), TNF-α was measured with a sandwich enzyme-linked immunosorbent assay (ELISA) using monoclonal antibodies raised against recombinant TNF-α (normal values <40 pg/ml), IL-6 was measured by an ELISA with a lower detection limit of <3 pg/ml, and IL-8 was measured by an ELISA in which antibodies to human recombinant IL-8 were used, the lower detection limit being <5 pg/ml. The intra-assay and interassay variation coefficients for the assays were <15%. Plasma elastase-α1-antitrypsin was measured using a radioimmunooassay and referenced to preformed complexes (normal values <100 ng/ml).

STATISTICAL ANALYSIS

Changes within groups were measured using the Wilcoxon signed rank test, and the Wilcoxon rank sum test was used to compare groups. For correlations, the rank correlation coefficient (rS) was used. A p value of <0.05 was considered statistically significant. Data are expressed as median and range, or median and 95% confidence intervals (CI) as indicated. The upper 95% CI limit of baseline values was considered to represent the upper limit of normal.

PRESSURES, CHEST RADIOGRAPH AND GAS EXCHANGE

At the time of PLI measurement after surgery the median PCWP was 9 (range 5–15) and the MPAP was 20 (11–22) mmHg. In the two patients without a pulmonary artery catheter the CVP was 2 and 8 mmHg. The PCWP and MPAP did not differ between patients with and without a postoperative lung PLI above the upper baseline limit. There was no difference in these

Results

All patients survived to discharge from hospital, with a median stay on the ICU of 47 (20–360) hours and a total time on mechanical ventilation of 23 (5–69) hours. No patient developed ARDS or clinical signs of ischaemic colitis.

PROTEIN LEAK INDEX (PLI)

The PLI in the leg (figs 1 and 2) increased in each patient from a median of 0.3 (range -1.6 to 1.7) × 10⁻³/min before surgery to 5.0 (1.0 to 27.8) × 10⁻³/min after surgery (p <0.005), with a median increase of 4.3 (95% CI 3.5 to 17.6) × 10⁻³/min. All postoperative values exceeded the upper limit of baseline values of 0.9 × 10⁻³/min. In the lung the PLI rose from a median of 0.6 (range -0.5 to 2.2) to 5.4 (± 2.3 to 33.5) × 10⁻³/min (p <0.02), with a median increase of 5.1 (95% CI 1.6 to 18.2) × 10⁻³/min. For both the PLI in the leg and the lung these results did not seem to relate to the type of surgery undertaken. The postoperative PLI exceeded the upper baseline limit (1.2 × 10⁻³/min) in seven patients. Leg and lung PLI did not significantly differ. The changes in the lung PLI correlated with those in the leg PLI (fig 2). There was no relation to the duration of aortic clamping.

Figure 1 Protein leak index (PLI) in (A) the leg and (B) the lung before and after aortic surgery. Postoperative versus preoperative values in the leg, p <0.005; in the lung p <0.02.

Figure 2 Relation between increases in leg and lung protein leak index (PLI): rS = 0.71, p <0.02. + = aneurysmatic aortic disease; ■ = occlusive aortoiliac disease.

Figure 3 Increase in leg PLI (10⁻³/min)
Permeability after aortic surgery

### Table 1 Course of respiratory variables after aortic surgery

<table>
<thead>
<tr>
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<th>Day 1</th>
<th>Day 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mechanical ventilation (n)</td>
<td>11</td>
<td>8</td>
</tr>
<tr>
<td>Positive end expiratory pressure (cm H2O)</td>
<td>5 (0 to 13)</td>
<td>5 (0 to 7)†</td>
</tr>
<tr>
<td>Inspiratory O2 fraction</td>
<td>0.40 (0.35 to 0.51)</td>
<td>0.40 (0.35 to 0.50)</td>
</tr>
<tr>
<td>Arterial Po2 (torr)</td>
<td>112 (85 to 179)</td>
<td>98 (70 to 139) **</td>
</tr>
<tr>
<td>Arterial Po2/inspiratory O2 fraction</td>
<td>292 (182 to 436)</td>
<td>232 (159 to 347) *</td>
</tr>
<tr>
<td>Interstitial oedema (n)</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td>Alveolar oedema (n)</td>
<td>1</td>
<td>2†</td>
</tr>
</tbody>
</table>

1 At time of postoperative protein leak index measurement.
2 1 torr = 0.133 kPa.
3 On chest radiograph.
4 In mechanically ventilated patients
5 p <0.05, ** p <0.005 versus day 0.

### Table 2 Cellular and humoral mediators

<table>
<thead>
<tr>
<th></th>
<th>Before surgery</th>
<th>Day 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leucocytes (x 10^9/l)</td>
<td>8.8 (7.1–119)</td>
<td>10.6 (6.9–17.8)</td>
</tr>
<tr>
<td>Neutrophils (x 10^9/l)</td>
<td>5.9 (4.0–8.3)</td>
<td>8.4 (4.9–16.6)*</td>
</tr>
<tr>
<td>Complement C3a (nmol/l)</td>
<td>5.0 (1.8–21.0)</td>
<td>6.5 (4.2–18.0)</td>
</tr>
<tr>
<td>Tumour necrosis factor-α (pg/ml)</td>
<td>&lt;40 (&lt;40)</td>
<td>&lt;40 (&lt;40 to 96)</td>
</tr>
<tr>
<td>Interleukin-6 (pg/ml)</td>
<td>5 (3–10)</td>
<td>57 (20–934)**</td>
</tr>
<tr>
<td>Interleukin-8 (pg/ml)</td>
<td>5 (5–10)</td>
<td>118 (5–578)*</td>
</tr>
<tr>
<td>Elastase-α1-antitrypsin (ng/ml)</td>
<td>55 (33–132)</td>
<td>155 (69–387)**</td>
</tr>
</tbody>
</table>

1 At time of postoperative protein leak index measurement.
2 p <0.05, ** p <0.02 versus before surgery.

pressures or the lung PLI in patients with radiographic evidence of pulmonary oedema compared with those without on day 0 or 1 (table 1). In only one patient with radiographic evidence of interstitial pulmonary oedema on day 1 did the lung PLI not increase (from 0.6 to 0.5 × 10^-14) min after surgery and the PCWP was 15 torr, the highest value recorded in this series of patients. The oxygenation ratio decreased from day 0 to 1, concomitantly with a fall in the median PEEP level and an increase in the number of patients with radiographic evidence of pulmonary oedema (table 1). The oxygenation ratio was lower in patients with radiographic evidence of pulmonary oedema than those without it on day 0 (p <0.05) so that the ratio was inversely related to the radiographic score (rS = -0.72, p <0.02). Nevertheless, three patients had been extubated on day 0 and five more on day 1. Neither the oxygenation ratio, the PEEP level, nor the radiographic score on days 0 or 1 were related to the increase in lung PLI.

**Discussion**

The results of this study suggest increased microvascular protein permeability in the leg and lung early after aortic surgery and I/R. The leg I/R may result in neutrophil activation and release of IL-8, which may induce neutrophil sequestration and subsequently increased microvascular permeability in the lung. Together with pressure factors, the latter might contribute to some degree of acute lung injury, pulmonary oedema, and gas exchange abnormalities after aortic surgery.

Our normal baseline values for the pulmonary 111In PLI agree with the literature.19 10 The normal pulmonary 67Ga PLI may be up to tenfold higher, however.5 The lower 111In than 67Ga PLI is probably caused by more firm binding of 111In to transferrin.9 9 The difference between radionuclides may partly explain the fact that aortic surgery increased the pulmonary 67Ga PLI in all patients in our previous study6 and the 111In PLI in only seven of the 11 patients in the current study. Nevertheless, the increases in 111In PLI after surgery fall in the range of that in patients with ARDS or those at risk for the syndrome.9 10 However, only a few patients developed evidence of alveolar pulmonary oedema and none fulfilled the clinical criteria for ARDS, so the predictive value of a raised 111In PLI is obscure.4 10 16 21 In a previous study increased permeability of the lungs (67Ga PLI) had returned to normal about 24 hours after aortic surgery.4 Hence, the increase in 111In PLI may have been transient or increased lymph flow may have offset increased protein permeability, thereby preventing development of overt alveolar pulmonary oedema in most of our patients.

The idea that the index is specific for protein permeability is supported by the lack of dependency of the lung 111In PLI on pressure factors.4 5 10 The absence of a relation between either pressure factors or permeability on the one hand and pulmonary oedema on the other...
may suggest that both factors contributed to the radiographic changes seen in some of our patients after surgery. In the only patient with radiographic evidence of interstitial pulmonary oedema at an unchanged lung PLI after surgery the PCWP was relatively high, suggesting that pressure factors rather than increased permeability were responsible. In any case, the development of pulmonary oedema contributed to impaired gas exchange after surgery.

Furthermore, our results suggest that the increased microvascular protein permeability in the lungs relates to the severity of I/R injury in the leg. Moreover, the former may not increase if the latter is relatively mild. Increased leg microvascular protein permeability after major vascular surgery may explain the clinical impression that successful surgical revascularisation of ischaemic limbs is associated with transient oedema, redness and pain, probably caused by I/R associated vascular injury. Indeed, animal experiments indicate that I/R of hindlimbs and skeletal muscle elicits (transient) microvascular changes with increased permeability to proteins, tissue oedema, and injury, which, in turn, promotes downstream sequestration of activated neutrophils and thereby to increased microvascular permeability and extravasation in local inflammatory sites. This may be at variance with the central role of activated neutrophils to I/R injury of hindlimb skeletal muscle in some animal studies, but agrees with studies showing that neutrophils hardly accumulate in skeletal muscle and only partly contribute to tissue injury after I/R. This may leave xanthine oxidase generated oxygen free radicals and eicosanoids as primary mediators of both the initial neutrophil activation and permeability increases in the leg after I/R.

Our study accords with previous observations that elective aortic surgery does not cause pulmonary oedema, apparently, not with transient oedema, redness and pain, probably caused by I/R associated vascular injury. Indeed, animal experiments indicate that I/R of hindlimbs and skeletal muscle elicits (transient) microvascular changes with increased permeability to proteins, tissue oedema, and injury, which, in turn, promotes downstream sequestration of activated neutrophils and thereby to increased microvascular permeability and extravasation in local inflammatory sites. This may be at variance with the central role of activated neutrophils to I/R injury of hindlimb skeletal muscle in some animal studies, but agrees with studies showing that neutrophils hardly accumulate in skeletal muscle and only partly contribute to tissue injury after I/R. This may leave xanthine oxidase generated oxygen free radicals and eicosanoids as primary mediators of both the initial neutrophil activation and permeability increases in the leg after I/R.

The increased levels of elastase-α1-antitrypsin in IL-8 may suggest neutrophil activation and IL-8 release by the I/R tissue. The release of IL-8 agrees with the literature, suggesting that this cytokine may have a role in the development of acute lung injury and ARDS in man. The data also agree with in vitro studies, showing that hypoxia/reperfusion increases IL-8 production by mononuclear and endothelial cells independently of TNF-α and IL-1. The release of IL-8 may have been caused by oxygen radicals produced by reperfusion induced xanthine oxidase since oxygen radical scavengers selectively inhibit IL-8 release by stimulated monocytes. Although IL-8 may be a potent activator of neutrophils, this effect may be limited to preactivated neutrophils. This might explain the lack of relation between IL-8 and elastase-α1-antitrypsin levels in our patients, suggesting that IL-8 may not have been the sole neutrophil activator. The direct relation between postoperative IL-8 levels and the PLI, and the inverse relation of these factors to leucocyte counts, are consistent with the idea that IL-8, released by the I/R tissue, contributes to neutrophil sequestration and thereby to increased microvascular permeability in the lungs. Indeed, the neutrophil may be central to the development of remote (in the lung, for example) microvascular injury after I/R in animals. Even though increases in lung PLI did not relate to circulating levels of elastase-α1-antitrypsin, the relation of the latter to the abnormalities on the chest radiograph may suggest that neutrophil derived products are involved in acute lung injury after I/R in man, in accordance with the literature.

The lack of a relationship between the leg PLI and circulating levels of leucocytes, elastase-α1-antitrypsin, and IL-8 after aortic surgery may be explained by experimental studies which have shown that intravenously injected IL-8 ameliorates neutrophil adherence and extravasation in local inflammatory sites. This is probably caused by stiffening of the neutrophil which, in turn, promotes downstream sequestration of the cells in the pulmonary microvasculature. Hence, activated neutrophils may have only partly contributed to the increased leg PLI after I/R induced by aortic surgery. This may be at variance with the central role of activated neutrophils to I/R injury of hindlimb skeletal muscle in some animal studies, but agrees with studies showing that neutrophils hardly accumulate in skeletal muscle and only partly contribute to tissue injury after I/R. This may leave xanthine oxidase generated oxygen free radicals and eicosanoids as primary mediators of both the initial neutrophil activation and permeability increases in the leg after I/R.

The limitations of the study include the relatively low number of patients and the use of baseline as the only control values. By specifically documenting increased microvascular protein permeability in both the leg and lungs after lower body I/R in man, our study nevertheless extends previous findings of the increase in 67Ga permeability in the lungs shortly after aortic surgery. The increased leg PLI after I/R induced by aortic surgery may explain the lack of relation between IL-8 and the PLI in the lungs early after peripheral vascular surgery in that study suggests that the increases in PLI seen early after aortic surgery in the current study are caused by extensive I/R rather than by the surgical procedures.

In conclusion, increased microvascular protein permeability in the lung after I/R associated with aortic surgery may be caused by pulmonary sequestration of activated neutrophils induced by IL-8 release upon I/R. Although none of the patients developed ARDS, the mechanisms proposed may help to explain the development of acute lung injury after major vascular surgery.

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