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## Consumption of Mannan-binding Lectin During Abdominal Aortic Aneurysm Repair

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**Objective.** Patients undergoing abdominal aortic aneurysm (AAA) repair are exposed to an ischaemia-reperfusion injury (IRI), which is in part mediated by complement activation. We investigated the role of the novel lectin pathway of complement during IRI in patients undergoing AAA repair.

**Methods.** Patients undergoing elective open infrarenal AAA repair had systemic blood samples taken at induction of anaesthesia, prior to aortic clamping, prior to aortic declamping and at reperfusion. Control patients undergoing major abdominal surgery were also included. Plasma was assayed for levels of mannan-binding lectin (MBL) using ELISA techniques. Consumption of plasma MBL was used as a measure of lectin pathway activation.

**Results.** Twenty-three patients undergoing AAA repair and eight control patients were recruited. No lectin pathway activation could be demonstrated in the control patients. AAA patients experienced a mean decrease in plasma MBL levels of 41% representing significant lectin pathway activation ( $p=0.003$ ).

**Conclusion.** Consumption of MBL occurs during AAA repair, suggesting an important role for the lectin pathway in IRI. Specific transient inhibition of lectin pathway activity could be of significant therapeutic value in patients undergoing open surgical AAA repair.

**Keywords:** Aortic aneurysm, Abdominal; Mannan-binding lectin; Reperfusion injury.

### Introduction

Abdominal aortic aneurysm (AAA) repair is associated with an inflammatory response,<sup>1</sup> which is thought in part to originate as a result of an ischaemia-reperfusion injury (IRI), secondary to aortic clamping. This response is mediated via several pathways, resulting in the production of a number of components of the inflammatory response including cytokines,<sup>2</sup> lipid-derived mediators<sup>3</sup> and complement components.<sup>4</sup> Excessive inflammation can lead to systemic effects (the systemic inflammatory response syndrome or SIRS),<sup>5</sup> and if severe may progress to organ failure.<sup>6</sup> Once established, organ failure has an appreciable mortality.<sup>7</sup>

Activation of the complement system has been demonstrated to occur as a result of IRI, and has specifically been demonstrated to occur during AAA repair.<sup>4,8–10</sup> The complement system may be activated

via three pathways; the classical pathway, the alternative pathway and the lectin pathway. The lectin pathway is activated when specific recognition complexes (comprising either MBL, H-ficolin or L-ficolin, complexed with the MBL-associated serine proteases 1–3) bind to effector molecules.<sup>11</sup> These effector molecules (cell surface carbohydrate ligands) were originally detected on bacterial cell walls, and the lectin pathway has been demonstrated to be important in defence against infection. More recently however, activation of the lectin pathway has been demonstrated to occur on contact with ischaemic endothelial cells. Activation of the lectin pathway is accompanied by consumption of all components involved, including the initiating recognition complexes.

Traditionally, the classical pathway has been assumed responsible for IRI-mediated complement activation.<sup>12</sup> However, recent research using murine models of IRI indicates that the lectin pathway is also activated during IRI.<sup>13</sup> The aim of this study was to investigate whether consumption of lectin pathway activation complexes occurs in patients undergoing AAA repair.

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## Methods

Consecutive patients undergoing elective, transperitoneal repair of infrarenal AAA were recruited. Ethical approval was granted by the local Ethics Committee. All patients gave informed consent, and all procedures were followed in accordance with institutional guidelines. Subjects that were MBL-deficient (serum MBL < 50 ng/ml) were excluded from this study. Arterial blood samples were taken from a radial artery line, which was sited at the time of induction of anaesthesia. Blood samples were taken at four defined time points during the procedure (time point 1: induction of anaesthesia, time point 2: just prior to aortic clamping, time point 3: just prior to aortic clamp removal and time point 4: after 30 min of reperfusion). Samples were taken using a sterile syringe, and the blood was transferred to 7 ml sterile glass EDTA tubes (Becton Dickinson, UK) and immediately placed on ice until the end of the operation, when samples were centrifuged at 2220 g for 10 min at 4 °C. Plasma was snap frozen for subsequent batch analysis. For comparison, control patients undergoing surgery to the pancreas and biliary tree were also recruited. Control patients had radial artery blood taken at two defined time points during the procedure (time point 1: induction of anaesthesia, time point 2: 2 h after the start of the procedure). Time point 2 corresponded with the reperfusion sample (time point 4) from AAA patients.

MBL levels were quantified using a modification of the method described by Haurum *et al.*<sup>13</sup> Briefly; Nunc Maxisorb microtiter plates (Nalge Nunc International, Rochester, NY) were coated with 1 µg/well of mannan (Sigma-Aldrich, St Louis, MO) in 15 mM Na<sub>2</sub>CO<sub>3</sub>, 35 mM NaHCO<sub>3</sub>, pH 9.6. Wells were blocked with 0.1% HSA in TBS (10 mM Tris-Cl, 140 mM NaCl, pH 7.4), then washed with TBS containing 0.05% Tween 20 and 5 mM CaCl<sub>2</sub> (wash buffer). Serum samples were diluted in 20 mM Tris-Cl, 1 M NaCl, 10 mM CaCl<sub>2</sub>, 0.05% Triton X-100, 0.1% HSA, pH 7.4, added to the plates and incubated overnight at 4 °C. After washing, bound MBL was detected using the mouse anti-human MBL mAb, HYB-131-01 (Antibody Shop, Copenhagen, Denmark), followed by alkaline phosphatase-conjugated goat anti-mouse IgG (Sigma-Aldrich) and the colorimetric substrate *p*-nitrophenyl phosphate (also from Sigma-Aldrich). The MBL concentration was calculated from a standard curve performed with each assay.

Statistical analysis was performed using SPSS v11.1 and STATA v8. Following assessment of normality of the data, descriptive statistics were expressed as the mean and standard deviation (SD) for normally distributed

data and by median value and inter-quartile range (IQR) for non-normally distributed data. MBL values were normally distributed, and expressed using the mean and standard error (SE) of the mean. Analysis of the changes in MBL levels within patients were performed using paired *t*-tests. A comparison of MBL levels between groups after reperfusion was made on the percentage change in levels from induction (to take into account differences between subjects at induction) using an (unpaired) *t*-test.

## Results

Twenty-three patients undergoing AAA repair and eight controls were recruited. The patient demographics are given in Table 1. The operative details are given in Table 2. All AAA patients underwent open, transperitoneal repair of AAA using a midline incision. All patients had infrarenal aortic clamp placement. 14 (61%) of patients received a straight aortic graft, with the remainder receiving bifurcated aorto-iliac grafts.

At induction of anaesthesia, the mean plasma MBL concentration was 3240 ng/ml (SE 510 ng/ml) in AAA patients, and 3600 ng/ml (SE 1100 ng/ml) in the controls ( $p=0.77$  Student's *t*-test, 95% CI for the difference -2335 to 3056). During AAA repair, patients demonstrated a steady decrease in plasma MBL reaching a mean minimum level of 1956 ng/ml (SE 397 ng/ml) after 30 min of reperfusion ( $p<0.01$  paired *t*-test, 95% CI for the difference 776-1811). In comparison to AAA patients, controls also experienced a slight decrease in their MBL levels, but this was not significant ( $p=0.07$  paired *t*-test, 95% CI for the difference -84 to 747) (Fig. 1).

To account for the fact that patients displayed a relatively large range of preoperative MBL levels, we also calculated the degree to which the MBL levels

**Table 1. Demographic data for all AAA subjects and controls**

	AAA patients ( <i>n</i> =23)	Controls ( <i>n</i> =8)
Mean age (S.D.)	67.74 (6.17)	54.75 (10.63)
Sex	♂=20 (87%)	♂=4 (50%)
Smoking history	Yes=9 (39.1%) Ex=12 (52.2%) Non=2 (8.7%)	Yes=3 (37.5%) Ex=1 (12.5%) No=4 (50%)
Hypertension	16 (69.6%)	3 (37.5%)
Ischaemic heart disease	8 (34.8%)	1 (12.5%)
Peripheral vascular disease	4 (17.4%)	0
Cerebrovascular disease	3 (13.0%)	0
Diabetes	4 (17.4%)	1 (12.5%)

Results are displayed as the number of patients, with percentage values given in brackets.

**Table 2. Operative and postoperative data for all AAA subjects and controls**

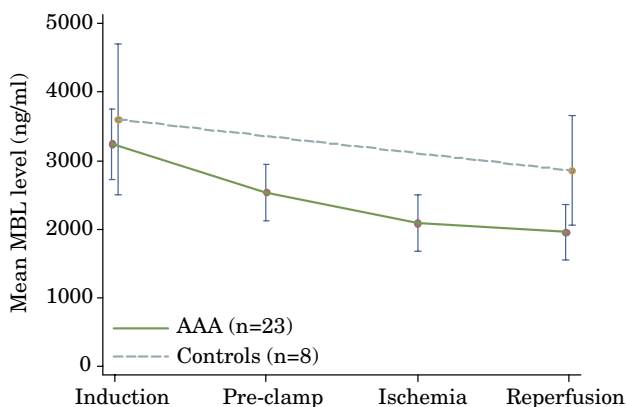
	AAA patients (n=23)	Controls (n=8)
Operation time (min)	162 (53)	271 (156)
Clamp time (min)	49 (12)	N/A
Straight graft	14 (61%)	N/A
Blood loss (ml)*	1000 (800–1600)	135 (100–500)
Blood transfusion (units)	2.17 (2.23)	1.0 (1.41)
Fluid replacement (l)*	5.5 (4.5–6.0)	5.5 (3.8–7.4)
POSSUM score†	36.26 (5.67)	31.0 (4.63)
ITU/HDU stay (days)*	1.0 (1.0–3.0)	1.0 (0.25–2.75)
Hospital stay (days)	9.0 (8.0–12.0)	9.5 (9.0–19.25)
Mortality	1 (4.3%)	0 (0%)

\* Results are displayed as the mean value (SD) unless the data is non-parametric, whereby the median value (IQR) is given.

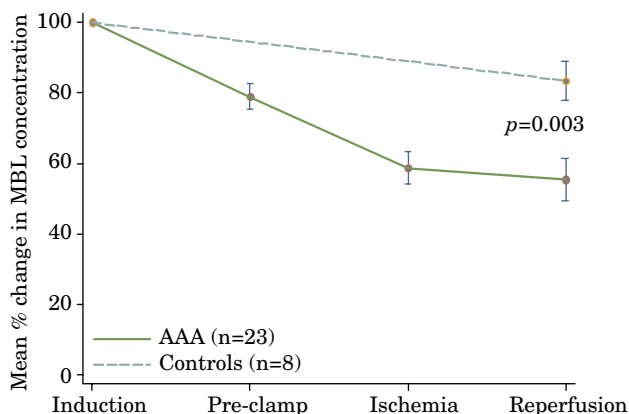
† POSSUM score (post operative surgical score for the enumeration of mortality) as described by Copeland *et al.*<sup>20</sup>

changed in each individual patient. This was achieved by dividing the pre-clamp, ischaemia and reperfusion values by the induction value, and hence displaying the values as the percentage change from baseline. Fig. 2 clearly demonstrates how MBL levels decrease during the procedure. AAA patients on average experienced a 41% (SE 4.5%) decrease in their MBL levels, whereas controls only experienced a 17% (SE 5.6%) decrease in MBL levels—a difference of 24%. This difference in the percentage change in MBL levels between AAA and control patients was statistically significant ( $p=0.003$ , 95% CI for the difference 9.5–40%, Student’s *t*-test). There were no differences in MBL levels between patients that had received a bifurcated graft rather than a straight graft.

Mortality was 0% in the control group and 4.3% ( $n=1$ ) in the AAA group. The cause of death was multiple organ failure. This patient did not demonstrate exceptional changes in MBL levels.



**Fig. 1.** Mean (standard error) change in systemic MBL levels (ng/ml) for AAA and control subjects at each stage of the procedure. For control subjects, the second blood sample was taken 2 h after the start of the procedure. This corresponds chronologically with the reperfusion value in the AAA group.



**Fig. 2.** Mean percentage (%) change (and standard error) in MBL levels for AAA ( $n=23$ ) and control ( $n=8$ ) subjects at each stage of the procedure. Starting values for MBL are 100%, with relative decreases thereafter. For control subjects, the corresponding reperfusion value is 2 h after the start of the procedure.

### Discussion

This is the first study to demonstrate consumption of MBL during elective, open repair of AAA. The complement system plays an essential role in IRI, and inhibition of complement activation reduces tissue damage in experimental models of myocardial ischaemia. Prior to the discovery of the lectin pathway, these effects were attributed to the classical pathway (mainly because cleavage of complement C4 was observed).<sup>12</sup> However, recent evidence indicates that it is the lectin pathway that mediates tissue damage in IRI. For example, in an *in vitro* study of endothelial cell oxidative stress, it was shown that MBL binds to ischaemic human endothelial cells (HUVECs) and that this binding can be inhibited by monoclonal antibodies against MBL<sup>14</sup>. Jordan *et al.* demonstrated that the administration of such antibodies also leads to a significant reduction in tissue injury in an *in vivo* model of myocardial infarction in rats<sup>15</sup>. Moreover, it has been shown that C1q deficient mice (i.e. mice deficient of the classical pathway) are not protected from complement mediated tissue damage in experimental models of gastrointestinal or myocardial ischemia,<sup>16,17</sup> whereas MBL deficient mice are protected.<sup>18</sup>

In a previous study monitoring complement activation in blood samples from subjects undergoing repair of thoraco-abdominal aortic aneurysm (TAAA) and using AAA subjects as controls, Fiane *et al.* observed downstream complement activation products (C4bc, C3bBbP and C5-9) in 16 MBL-sufficient TAAA patients, and in two MBL-deficient patients that had received plasma during surgery, but

none in an MBL-deficient patient that had not received plasma.<sup>19</sup> The authors therefore concluded that MBL-mediated complement activation is responsible for the generation of downstream complement activation products during TAAA repair. In contrast to our results, Fiane and co-workers did not observe a consumption of MBL during the course of surgery. The precise reason for this is unclear, but may be a reflection of ELISA technique (whether plasma or serum was used for the ELISA was not specified), data analysis, operation time (this was not specified) or sample size (only five AAA patients were used, two of which were MBL deficient).

Our data clearly demonstrate a significant drop in MBL levels in all 23 patients during AAA. The slight decrease in MBL levels observed between time points 1 and 2 (after induction of anaesthesia and just prior to aortic clamping) cannot be accounted for by IRI, but it was of a similar magnitude to the total decrease seen in the controls, and is therefore likely to be a consequence of the surgical procedure per se. The consumption of MBL between time points 2 and 4 was highly significant, and is indicative of MBL deposition, presumably on the damaged endothelium. These findings add to a growing body of evidence that supports an important role for the lectin pathway in IRI. Further studies would be useful however using regional blood sampling techniques (as used in previous studies<sup>21-23</sup>) to attempt to elicit the origin of this inflammatory response. An older control group would ideally also be used to address the age difference between AAA patients and controls in this study.

In light of the well established contribution of complement activation to the pathology of reperfusion injury, and the rapidly accumulating evidence that the lectin pathway is activated on ischaemic endothelial cells, we suggest that a transient inhibition of lectin pathway activity could be a potential therapeutic target to improve outcomes in patients undergoing AAA surgery. Furthermore, transient inhibition of lectin pathway activity could have a significant therapeutic impact on the outcome of other diseases or procedures that involve a transient ischaemic insult, i.e. myocardial infarction, gut infarction, burns, transplantation and stroke.

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#### References

- SWARTBOL P, TRUEDSSON L, NORNGREN L. The inflammatory response and its consequence for the clinical outcome following aortic aneurysm repair. *Eur J Vasc Endovasc Surg* 2001;**21**:393-400.
- ROWLANDS TE, HOMER-VANNIASINKAM S. Pro- and anti-inflammatory cytokine release in open versus endovascular repair of AAA. *Br J Surg* 2001;**88**:1335-1340.
- LAMMERS KM, INNOCENTI G, VENTURI A, RIZZELLO F, HELWIG U, BIANCHI GP *et al.* The effect of transient intestinal ischaemia on inflammatory parameters. *Int J Colorectal Dis* 2003;**18**:78-85.
- BENGTSON A, LANNSJO W, HEIDEMAN M. Complement and anaphylatoxin responses to cross-clamping of the aorta. *Br J Anaesth* 1987;**59**:1093-1097.
- BEAL AL, CERRA FB. Multiple organ failure syndrome in the 1990s. Systemic inflammatory response and organ dysfunction. *JAMA* 1994;**271**:226-233.
- BOWN MJ, NICHOLSON ML, BELL PRF, SAYERS RD. The systemic inflammatory response syndrome (SIRS), organ failure, and mortality after abdominal aortic aneurysm repair. *J Vasc Surg* 2003;**37**:600-606.
- SAYERS RD, THOMPSON MM, NASIM A, HEALEY P, TAUB N, BELL PR. Surgical management of 671 abdominal aortic aneurysms: a 13-year review from a single centre. *Eur J Vasc Endovasc Surg* 1997;**13**:322-327.
- GALLE C, DE MAERTELAER V, MOTTE S, ZHOU L, STORDEUR P, DELVILLE JP *et al.* Early inflammatory responses after elective abdominal aortic aneurysm repair: a comparison between endovascular procedure and conventional surgery. *J Vasc Surg* 2000;**32**:234-246.
- FOSSE E, OPDAHL H, SUNDE S, AASEN AO, SVENNEVIG JL. Granulocytes in bronchial lavage fluid after major vascular surgery. *Acta Anaesthesiol Scand* 1987;**31**:33-37.
- SWARTBOL P, NORNGREN L, ALBRECHTSSON U, CWIKIEL W, JAHR J, JONUNG T *et al.* Biological responses differ considerably between endovascular and conventional aortic aneurysm surgery. *Eur J Vasc Endovasc Surg* 1996;**12**:18-25.
- SCHWAEBLE WJ, DAHL MR, THIEL S, STOVER C, JENSENIUS JC. The mannan-binding lectin-associated serine proteases (MASPs) and MASP19: four components of the lectin pathway activation complex encoded by two genes. *Immunobiol* 2002;**205**:455-466.
- ZHANG M, AUSTIN Jr WG, CHIU I, ALICOT EM, HUNG R, MA M *et al.* Identification of a specific self-reactive IgM antibody that initiates intestinal ischaemia/reperfusion injury. *Proc Natl Acad Sci* 2004;**101**:3886-3891.
- HAURUM JS, THIEL S, HAAGSMAN HP, LAURSEN SB, LARSEN B, JENSENIUS JC. Studies on the carbohydrate-binding characteristics of human pulmonary surfactant-associated protein A and comparison with two other collectins: mannan-binding protein and conglutinin. *Biochem J* 1993;**293**:873.
- COLLARD CD, VAKEVA A, MORRISSEY MA, AGAH A, ROLLINS SA, REENSTRA WR *et al.* Complement activation after oxidative stress: role of the lectin complement pathway. *Am J Pathol* 2000;**156**:1549-1556.
- JORDAN JE, MONTALTO MC, STAHL GL. Inhibition of mannose-binding lectin reduces postischemic myocardial reperfusion injury. *Circulation* 2001;**104**:1413-1418.
- HART ML, CEONZO KA, BURAS JA, STAHL GL. Gastrointestinal ischaemia-reperfusion injury is complement-dependent but not dependent on C1q. *Mol Immunol* 2003;**40**:187.
- WALSH MC, BOURCIER T, TAKAHASHI M, STAHL GM. Complement activation and tissue injury following myocardial ischaemia and reperfusion is C1q independent. *Mol Immunol* 2003;**40**:215.
- WALSH MC, TAKAHASHI K, SHI L, ROTHER R, BOURGON M, SOLOMON SD *et al.* Complement activation and tissue injury following myocardial ischemia and reperfusion is dependent on MBL. In Abstracts from the XXth International Complement Workshop. *Mol Immunol* 2004;**41**:322.
- FIANE AE, VIDEM V, LINGAAS PS, HEGGELUND L, NIELSEN EW, GEIRAN OR *et al.* Mechanism of complement activation and its

- role in the inflammatory response after thoracoabdominal aortic aneurysm repair. *Circulation* 2003;**108**:849–856.
- 20 COPELAND GP, JONES D, WALTERS M. POSSUM: a scoring system for surgical audit. *Br J Surg* 1991;**78**:355–360.
- 21 NORWOOD MGA, BOWN MJ, HORSBURGH T, SAYERS RD. Neutrophil activation occurs in the lower-limbs of patients undergoing elective repair of abdominal aortic aneurysm. *Eur J Vasc Endovasc Surg* 2005;**29**:390–394.
- 22 NORWOOD MGA, BOWN MJ, SUTTON AJ, NICHOLSON ML, SAYERS RD. Interleukin-6 production during abdominal aortic aneurysm repair arises from the gastrointestinal tract and not the lower limbs. *Br J Surg* 2004;**91**:1153–1156.
- 23 NORWOOD MGA, BOWN MJ, SAYERS RD. Ischaemia reperfusion injury and regional inflammatory responses in abdominal aortic aneurysm repair. *Eur J Vasc Endovasc Surg* 2004;**28**:234–245.

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