The Effect of Acute Normovolaemic Haemodilution on the Inflammatory Response and Clinical Outcome in Abdominal Aortic Aneurysm Repair—Results of a Pilot Trial


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Objectives. To determine the effect of acute normovolaemic haemodilution (ANH) on the inflammatory response and clinical outcome in elective open abdominal aortic aneurysm (AAA) repair.

Design. Randomised controlled clinical trial.

Methods. Thirty-six patients were randomised to undergo ANH or act as controls. Cell salvage was permitted in both groups. Heterologous blood was transfused according to pre-determined triggers. Outcome measures were markers of the systemic inflammatory response in serum and urine observed at multiple time points, and clinical recovery.

Results. Median 890 (range 670–1620) ml of blood was removed at ANH in 16 patients. There were no differences in peri-operative changes in neutrophil count (P=0.13), serum C-reactive protein (P=0.38), interleukin-6 (P=0.50), total antioxidant capacity (P=0.73), urinary secretion of albumin (P=0.97) or retinol binding protein (P=0.41). There were no differences in the mortality and morbidity rates, systemic inflammatory response syndrome, ITU or hospital stay.

Conclusions. ANH, when used in combination with cell salvage, made no impact on systemic inflammatory response and clinical outcome when compared to cell salvage alone after AAA repair. ANH cannot be recommended for routine use in patients undergoing abdominal aortic aneurysm surgery when cell salvage is available.

Keywords: Acute normovolaemic haemodilution; Abdominal aortic aneurysm; Inflammatory response; Clinical outcome.

Peri-operative blood conservation is an important issue in modern surgical management. Acute normovolaemic haemodilution (ANH) has been proposed as a useful technique in reducing requirements for heterologous blood transfusion in a variety of surgical procedures but its clinical efficiency remains controversial.

Recent randomised clinical trials on patients undergoing elective open AAA repair with cell salvage showed no additional blood savings for ANH. At present this method is used only by enthusiasts. ANH could have other beneficial effects in major vascular surgery. Some studies on animal models showed that ANH can ameliorate ischaemia-reperfusion injury in the microcirculation of the skeletal muscle and a clinical study demonstrated that ANH preserves renal function and alleviates deleterious haemodynamic effects of aortic cross-clamping in patients undergoing abdominal aortic surgery.

This study investigates the impact of ANH on the inflammatory response and clinical outcome in a randomised controlled clinical trial on patients undergoing elective open AAA repair with cell salvage permitted in both groups.

Patients and Methods

Ethical approval was granted by the South West Local Research Ethics Committee. Forty-nine consecutive patients scheduled to undergo elective open repair of infra-renal AAA at the Bristol Royal Infirmary between July 1998 and August 2000 were considered for the trial. During this period 12 other patients with AAA were treated by endovascular stenting and were, therefore, not eligible to enter the trial. Exclusion criteria and methods including ANH procedure, cell...
salvage and indications for blood transfusion were described in our previous publication. Five patients were excluded and a further eight did not consent leaving 36 patients, who were randomised to undergo ANH or standard management using a computer generated randomisation grid in blocks of four. This study was not on intention-to-treat basis.

Assessment of systemic inflammatory response and clinical outcome

Various recognised inflammatory markers in the serum and urine were measured peri-operatively at defined time intervals to quantify the severity of surgical trauma and magnitude of the systemic inflammatory response. Polymorphonuclear neutrophils have been indicated to play a pivotal role in the pathophysiology of the local and systemic ischaemia-reperfusion injury.

Change in neutrophil count is a non-specific but simple marker of inflammatory response and has been used, among other parameters, to assess outcome in research and comparative studies on aortic surgery. C-reactive protein (CRP) is an acute-phase protein wildly used as an indicator of systemic response to inflammation. Although it is a non-specific marker, CRP is a simple and sensitive test and has been used to measure outcome in clinical studies on inflammatory response to abdominal aortic aneurysm surgery.

Interleukin-6 (IL-6) is one of the most important cytokines involved in mediation of the systemic response to tissue injury and inflammation and has been used as one of the principle research markers of systemic inflammatory response in AAA surgery. Depletion in the serum total antioxidant capacity (TAC) in vascular surgery has been described before and was found to correlate well with ischaemia-reperfusion injury in both AAA repair and bypass procedures for ischaemic limbs. Microalbuminuria is a sensitive non-specific marker of acute and chronic systemic disease, local and systemic inflammatory conditions, and ischaemia-reperfusion injury.

It reflects the renal glomerular component of the systemic endothelial damage triggered by ischaemia-reperfusion injury associated with aortic clamping and clamp release. Retinol binding protein (RBP) is a low molecular weight protein found in serum, secreted in the renal glomerulus and reabsorbed in the proximal tubule. Urinary excretion of retinol-binding protein is a marker of renal tubular damage cause by chronic pathological conditions affecting the kidneys and acute states characterised by systemic inflammatory response. The assessment of clinical outcome was based on the crude mortality and morbidity figures, the presence of the systemic inflammatory response syndrome (SIRS) as defined by ACCP/SCCM, and the ITU and hospital stay. Major complications were those, which required invasive treatment and monitoring on ITU. Minor complications, which were treated on the ward and did not delay hospital stay were not analysed. Patients with uncomplicated recovery were routinely observed overnight on ITU and discharged to the ward the next morning. Longer than 24-h ITU stay was considered as delayed.

Laboratory techniques

Blood samples for WCC were collected pre-operatively, 1 h post-operatively and then on day 1, 2, 3, 5 and 7 after surgery. The differential WCC was performed on the ADVIA 120 analyser (Bayer Diagnostics) using two separate methods to achieve the differentiation. The basophil (Baso) method provided the primary total WCC and used the resistance of basophils to acid lysis, which differentiates them from the rest of the white blood cell population. The peroxidase (Perox) method was the primary differential method, which is two-stage and uses three reagents to stain intra-cellular myeloperoxidase and then passes the cells through a flowcell where light scatter and absorption is used to determine each cell’s size and level of staining. The information was plotted on the cytogram; cluster analysis was then employed to determine the thresholds to separate the cell populations.

Venous blood for CRP, IL-6 and TAC (4 ml) were taken into the plain vacutainer tubes without additives. Samples were taken pre-operatively, pre-aortic cross-clamping and then 1, 6, 12, 24 h after aortic cross-clamping and on day 3 and day 7 after surgery. Blood was allowed to clot at room temperature for 20 min and then centrifuged at 3000 rpm for 10 min, serum aspirated into the cryogenic aliquots (Cellstar, Greiner Labortecnik) and stored in the −70°C freezer for further processing. Serum C-reactive protein was measured using a commercially available immuno-turbidimetric assay (Randox Laboratories Ltd, UK) on an Olympus AU600 autoanalyser (Olympus Instruments, UK). The assay uses a monoclonal antibody to CRP and its interaction with CRP is measured turbidimetrically at 340 nm. The values were expressed in mg/l. Serum interleukin-6 concentration was determined by the quantitative ‘sandwich’ enzyme immunoassay (ELISA) technique using commercially available kits (Biotrak human interleukin-6 [h]IL-6) ELISA system, code RPN 2754, Amersham.
Pharmacia Biotech, UK). Stored serum samples were defrosted and processed according to the manufacturer’s protocol. Optical density was determined spectrophotometrically using ELISA plate reader (Labsystems Multiscan Plus) and the IL-6 concentration read automatically from the standard curve using computer software (Genesis II Windows™ based microplate software, Labsystems and Life Sciences International (UK) Ltd). All measurements were carried out in duplicates and the mean value was taken. Serum IL-6 concentration was expressed in ng/l.

The IL-6 assay was performed by the main investigator in the laboratory of the Department of Surgery at the Bristol University and supervised by the laboratory technicians. Serum TAC was measured using a commercially available assay (Total Antioxidant Status, Randox Laboratories Ltd, UK) on a Cobas Mira analyser (Roche Laboratories Ltd, UK). The assay is based on the reaction between the radical cation ABTS<sup>•</sup> and serum antioxidants. ABTS (2,2'-Azino-di-[3-ethylbenzthiazoline sulphonate]) is incubated with a peroxidase (metmyoglobin) and H<sub>2</sub>O<sub>2</sub> to produce the radical cation ABTS<sup>••</sup>, which has a stable blue–green colour (measured at 600 nm). Antioxidants in the added sample cause suppression of this colour to a degree, which is proportional to their concentration. The assay is standardised using the synthetic antioxidant Trolox and the results expressed in mmol/l Trolox equivalents.

Urine for an albumin/creatinine and rbp/creatinine ratio (15–20 ml) was collected into sterile bottles either by the patient or from the urinary catheter, which was inserted in the anaesthetic room. Urine samples were collected pre-operatively, pre-aortic cross clamping, during aortic clamp, 30 min after aortic clamp release and then 2, 4, 6, 8, 12, 24 h and 3 days post-operatively. Samples were stored in the fridge in +4 °C until measurements according to the assay guidelines. Urinary albumin concentrations was measured using a commercially available immunoturbidimetric assay (Randox Laboratories Ldt, UK) and urinary creatinine by standard kinetic Jaffe method on a Kone Pro analyser (Labmedics, UK) in parallel with albumin. Commercially available microalbumin and creatinine controls (Randox Laboratories Ldt) were used and the measurements read on the Kone Pro analyser. Urinary concentrations of albumin were quoted as ratios to creatinine and expressed in g/μmol. Quantification of the urinary retinol binding protein was carried out by an ‘in-house’ ELISA assay. The plate was coated with polyclonal rabbit anti-human rbp antiserum. Diluted urine samples, standards and controls were applied to plate and incubated overnight. After washing, peroxidase conjugated rabbit anti-human rbp was added to the plate followed by substrate. Controls and samples were quantified by comparison with a standard curve. Urinary retinol-binding protein was expressed as ratio to creatinine and measured in g/μmol.

Statistical analysis

Populations of demographic data had normal or near-normal distribution (age, POSSUM, AAA size) and were expressed appropriately as mean (standard deviation) or median (range); comparative analysis was by parametric (Student’s t-test) or non-parametric (Mann–Whitney U test) test. Observations of outcome measures were taken at multiple time points. Histograms and Shapiro Wilk test for normality showed most of the variables were not normally distributed. Instead of analysing the data at each time point a summary measure of the individual data over time was analysed. The summary measure used was weighted mean, calculated by dividing the area under curve by the time period it was measured over, for each individual and each variable of interest. Area under curve was calculated using the linear trapezoid method and Mann–Whitney U test was used for analysis. Proportions were analysed using Chi-square and Fisher’s exact tests as appropriate. Peri-operative changes of Hb concentrations within each group (paired data) were analysed by Wilcoxon test. Results obtained from patients who died were included in the statistical analysis. P<0.05 was considered statistically significant.

Results

Patients, aneurysms and grafts

Both groups were well matched for age, gender, weight and pre-operative general health as assessed pre-operatively by the physiological part of the POSSUM score. In the whole cohort there were nine active cigarettes smokers, 24 ex-smokers and three patients who never smoked (equally distributed in the two groups). All AAAs were infra-renal. Supra-renal clamping requirement and the number of straight and bifurcated grafts inserted were similar in each group (Table 1).
Acute normovolaemic haemodilution, blood loss, cell salvage and blood transfusion

Two patients randomised into the experimental group did not have ANH and were excluded from further analysis. Sixteen patients underwent withdrawal of a mean 990 (SD 256, median 890, range 670–1620) ml of blood, which was retransfused during or shortly after surgery. Surgical blood loss, intra-operative cell salvage, volumes of intravenous fluids and the requirements for heterologous blood transfusion were similar in both groups. These results were published in detail previously.3

Systemic inflammatory response and clinical outcome

In most patients white cell count (WCC) increased 1 h post-operatively and highest values were observed on the 2nd day after surgery. A second rise was noted at 7 days post-operatively (Fig. 1). The peri-operative changes of WCC did not differ between the two groups: median (interquartile range) of 9.1 (8.8–12.8) × 10⁹/l in the ANH group versus 8.9 (7.8–9.6) × 10⁹/l in the control group (Mann–Whitney, Z = 1.52, P = 0.13). The changes in the neutrophil count (Fig. 2) paralleled the changes in WCC: median (interquartile range) of 7.2 (6.9–10.6) × 10⁹/l in the ANH group versus 7.0 (6.2–7.4) × 10⁹/l in the control group (Z = −1.51, P = 0.13).

An initial drop in the serum concentration of C-reactive protein from the pre-operative values occurred before application of the aortic clamp and 1 h after clamp release. The concentration was only mildly elevated at 6 h after aortic clamp release, then increased steadily achieving maximum values at 3 days (several-fold rise from pre-op) and remained significantly elevated at 7 days post-operatively (Fig. 3). There were large ranges in values at each time point between individual patients. The changes of C-reactive protein did not differ between the two groups: median (interquartile range) 93.1 (44.1–99.2) mg/l in the ANH versus 119.9 (70.7–134.3) mg/l in the control group (Mann–Whitney U test, Z = 0.88, P = 0.38).

The baseline serum Il-6 concentrations were low. An increase was observed at 1 h after aortic clamp release. In the majority of patients, the concentration peaked at 6 h after clamp release, remained elevated until 24 h, decreased by day 3 and remained raised at 7 days post-operatively (Fig. 4). Three patients who suffered serious complications (bleeding leading to multiorgan failure, leg ischaemia and non-fatal

**Table 1. Demographics**

<table>
<thead>
<tr>
<th></th>
<th>ANH group (n = 16)</th>
<th>Control group (n = 18)</th>
<th>Statistical test</th>
<th>P value</th>
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<tr>
<td>Age (years)</td>
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<tr>
<td>Median (range)</td>
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<td>76 (60–87)</td>
<td>Mann–Whitney</td>
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<td>Female:male</td>
<td>6:10</td>
<td>5:13</td>
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<td>Weight (kg)</td>
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<tr>
<td>Median (range)</td>
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<td>78 (57–105)</td>
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<tr>
<td>Media (range)</td>
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<td>18.5 (12–28)</td>
<td>Mann–Whitney</td>
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<td>AAA size (cm)</td>
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<td>Student's t test</td>
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<tr>
<td>Mean ± SD</td>
<td>5.9 ± 0.9</td>
<td>6.5 ± 0.9</td>
<td></td>
<td></td>
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<tr>
<td>Aortic clamp</td>
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<tr>
<td>Infrarenal:suprarenal</td>
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<td>15:3</td>
<td>Fisher's exact test</td>
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<td>Grafts</td>
<td>Straight:bifurcated</td>
<td>10:6</td>
<td>Chi-square test</td>
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myocardial infarct) developed unusually high IL-6 responses between 6 and 24 h post-operatively (509, 1590 and 793 ng/l, respectively). In all other patients, including another who had a fatal myocardial infarct, IL-6 concentrations did not rise above 270 ng/l. There was no evidence to suggest there was a difference in the distribution of IL-6 between patients in the two groups: median (interquartile range) of 44.1 (26.7–80.6) ng/l in the ANH group versus 53.3 (32.7–71.8) ng/l in the control group (Mann–Whitney U test, Z=0.68, P=0.50).

Serum total antioxidant capacity (TAC) decreased intra-operatively and remained low for 7 days post-operatively with a gradual recovery to the pre-operative levels (Fig. 5). The peri-operative changes of serum TAC did not differ between the two groups: median (interquartile range) of 0.944 (0.844–0.980) mmol/l in the ANH group versus 0.906 (0.902–0.968) mmol/l in the control group (Mann–Whitney U test, Z=−0.34, P=0.73).

Albuminuria increased during surgery achieving maximum urinary albumin/creatinine ratios within 2 h after aortic clamp release and then quickly decreased post-operatively (Fig. 6). Marked variation in absolute values between individual patients in both groups were observed at most time points. There was no statistical difference in the peri-operative albuminuria between the two groups: median (interquartile range) of 10.86 (4.32–29.13)×10^{-6} g/μmol in the ANH group versus 13.35 (6.96–29.80)×10^{-6} g/μmol in the control group (Mann–Whitney U test, Z=0.042, P=0.97).

The urinary excretion of retinol binding protein increased peri-operatively in all patients and remained elevated at 3 days post-operatively but there was no consistent time point for the peak values and the individual traces often followed a seesaw pattern (Fig. 7). There was no statistical difference in the peri-operative excretion of retinol binding protein between the two groups: median (interquartile range) of 6.28 (1.59–7.78)×10^{-6} g/μmol in the ANH group versus 2.66 (0.05–7.26)×10^{-6} g/μmol (Mann–Whitney U test, Z=−0.83, P=0.41).

There was one death (from multiorgan failure) and three major post-operative complications (two myocardial infarcts, one left ventricular failure) in the ANH group versus two deaths (one from myocardial infarct, one from leg ischaemia in a patient who refused further surgery) and four major complications (one respiratory failure, one acute renal failure, two acute left ventricular failures) in the control group (Fisher’s exact test, P=0.98). In the survivors SIRS was observed in the post-operative period in 10/15 in the
ANH and 11/16 patients in the control group (Chi-square test, \( P = 1.0 \)). The severity of symptoms varied between individuals and ranged from single episodes of tachycardia and tachypnoea to pyrexia > 38°C during otherwise uncomplicated post-operative recovery to prolonged periods of tachycardia associated with leukocytosis in patients with multiorgan failure.

In the ANH group 4/15 patients had prolonged ITU stay and one was readmitted to ITU from the ward. By comparison, in the control group 3/16 had prolonged ITU stay (including deaths) and the difference was statistically not significant (Chi-square test, \( P = 0.99 \)). The median (range, mean ± SD) hospital stay was 11 (7–22, 12 ± 4.4) days in the ANH group versus 13 (7–25, 12 ± 5.3) days in the control group and the difference was not significant (Mann–Whitney \( U \) test, \( P = 0.98 \)).

**Discussion**

In our previous study\(^3\) based on the same series of patients we showed that ANH in AAA surgery did not incur any demonstrable savings on the requirements for bank blood. We discussed possible reasons for this result. The important finding of this study is that ANH, when used in combination with cell salvage in open AAA repair, did not exert any demonstrable effect on the systemic inflammatory response and clinical outcome, when compared to cell salvage alone. This negative result may relate to similar requirements for heterologous blood transfusion in the two groups of patients. Small number of patients invalidated comparative analysis of subgroups. Large volumes of blood (mean 990 ml) were withdrawn at the ANH procedure to ensure significant impact on outcome measures. It is remarkable that the majority of patients in the experimental group tolerated the ANH procedure well. They were older than cohorts of patients in other reports on ANH during abdominal aortic\(^8\), cardiac\(^39\), or non-vascular surgery\(^40–42\) and many had cardiac comorbidities. Two patients became cardiovascularly unstable during ANH and we agree with previous suggestions\(^39,41,43\) that suitability to ANH should be based on the assessment of cardiovascular reserve rather than chronological age.

The general patterns of the peri-operative traces of the inflammatory markers were consistent with those reported in other studies on AAA repair\(^17,20,27,28,44,45\). Large variations were observed in the individual responses of serum IL-6, C-reactive protein, neutrophilia, and urinary excretion of albumin and retinol binding protein. This variability may result from the differences in the magnitude of surgical insult as well as the responsiveness of the host’s immune system. Urinary secretion of retinol binding protein is a recognised marker of ischaemia–reperfusion injury and has been used in research to evaluate renal damage in cardiac surgery\(^37\) and renal transplantation\(^36\), but, unlike albuminuria, has not been measured in AAA surgery before. Systemic inflammatory response syndrome was observed in two thirds of patients in both groups, which is higher than in other reports\(^46\) and the magnitude of clinical response varied markedly. We have previously highlighted that SIRS, while being a useful clinical indicator of the inflammatory response to an insult with a clear definition,\(^47\) needs to be developed into a more discriminatory system consisting of clinical and biochemical parameters if it is to be used as a valuable research tool\(^48\).

Our previous\(^3\) and present studies suggest that routine use of ANH does not save bank blood and does not improve the inflammatory response or clinical outcome in AAA surgery. These results are disappointing and do not support routine use of ANH in major vascular surgery, when cell salvage is available.

**References**

5. Menger MD, Sack FJ, Barker JH, Feifel G, Messmer K. Quantitative analysis of microcirculatory disorders after...


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