
In the US, H. pylori vacA shows allelic variation in the signal sequence (which may be type 1a, 1b or 2) and the mid-region (type ml or m2). Previous PCR-based vacA mid-region typing classified most, but not all, Asian and South American strains tested as m1 or m2. We now sought to investigate vacA mid-region diversity further.

Methods: We studied 13 Japanese, 6 Chinese, 9 Thai, and 8 Peruvian strains. H. pylori isolates were identified by colony hybridisation (CH), vacA signal sequence was typed by PCR, and vacA mid-region was typed proximally by CH and distally by PCR. Sections of the vacA mid-region from 8 strains were PCR-amplified, sequenced, and compared with known sequences from 8 other strains.

Results: Of the 36 Asian and South American strains studied, 35 were cagA+ (the cagA+ was Peruvian) and 35 were vacA 1a (1 vacA+ Peruvian was 1b). vacA mid-regions from the 13 Japanese strains were not PCR-amplified by ml or m2-specific primers, but hybridised weakly with an ml probe. Sequence analysis of vacA from 1 Japanese strain revealed 91% nucleotide identity with the ml probe but only 71% identity with the ml probe. The previously equivocal Thai and Peruvian strains also had ml-like mid-region sequences. A Chinese strain was ml in the proximal mid-region and ml2 distally, showing a clear crossover site. Final mid-region types were: Japanese, all 13 m1; Chinese, 1 m1, 1 m1/ml, 4 m2; Thai, 3 m1, 6 m2; Peruvian 4 m1, 4 m2. Distal mid-region sequences of 16 strains, compared over 294 bp, clustered into 2 groups, m1 and m2. Nucleotide identity between m1 and m2 strains ranged from 73-78%. Within groups, m2 strains were less diverse than m1 strains (m2 range 94-99.7%, m1 88-99.3%, p<0.001).

Conclusions: These Asian and South American strains are similar in terms of cagA status and vacA 1a genotype, but fall into 2 vacA mid-region groups, m1 and m2. The prevalance of vacA mid-region identity suggests recombination in vivo between m1 and m2 alleles.

HELCOBACTER PYLORI (H PYLORI) ANTIMICROBIAL RESISTANCE IN THE UK. QN Karim*, RPH Logaston†: the Glaxo Wellcome H pylori Study Group. * St Mary’s Hospital, Paddington. † University Hospital, Nottingham.

Introduction: H pylori antimicrobial susceptibility is an important determinant of the efficacy of eradication therapies. The prevalence of antimicrobial resistance varies within the UK and may increase given the increased use of eradication therapies.

Methods: Multicentre study assessed the prevalence and possible associations of H pylori antimicrobial resistance.

Results: H pylori was isolated from antral biopsies of patients undergoing routine endoscopy and cultured according to standard microbiological methods. Antimicrobial resistance was determined using "E-tests" or disc tests (tinzidazole only) with breakpoints defined by previous studies.

Results: H pylori was isolated from 32% (1222/3823) of patients and antimicrobial susceptibility determined in 90% (1077/1222) of positive cultures. The prevalence of resistance (median + ranges) for the most widely used antimicrobials are:

- Metronidazole:
  - Resistance: 38.6 (14.6-65.2)
  - Tetracycline:
    - Resistance: 38.6 (14.6-65.2)
  - Clarithromycin:
    - Resistance: 38.6 (14.6-65.2)

Conclusions: Resistance to metronidazole was more frequent in isolates from inner city centres (45% compared with those in rural centres (17.7%). This difference was statistically significant (P<0.001). Patients less than 40 years of age showed greater resistance to metronidazole than patients over 40 years (P=0.001), resistance being more prevalent in the female population (P=0.001). Over a 2 year period, there has been no change in the prevalence of metronidazole or clarithromycin resistance. Multiple resistance was seen in approximately 5% of isolates. The prevalence of antimicrobial resistance to H pylori does not appear to be increasing but varies with location, gender and age, predictors of metronidazole resistance. In addition, multiple antimicrobial resistance seen in approximately 5% of H pylori positive isolates, underlines the importance of establishing local patterns of antimicrobial resistance and selecting appropriate eradication regimens.


Inflammatory bowel disease T59-T70

CHILDHOOD RISK FACTORS FOR IBD USING A TWIN CASE-CONTROL METHOD

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Previous case-control studies of IBD have used hospital or community derived controls. There are obvious inherent biases in both methods, which also fail to control for genetic factors. Same-sex twin studies overcome both these flaws and are statistically more powerful.

Methods: Each member of a registry of 175 twins-pairs, at least one of which had IBD, was independently sent validated questionnaires confirming their disease, zygosity and asking about childhood (age <16 years old) risk factors.

Results: In 130 cases both twins replied, 116 discordant for the disease, 80 of which were the same gender (47 monozygotic). These 80 pairs formed the case-control study.

Risk factor | Case | Control | Odds ratio (95% CI)
--- | --- | --- | ---
Measles | 64 | 56 | 1.71 (0.8 - 3.8)
Mumps | 38 | 43 | 0.78 (0.4 - 1.5)
Chickenpox | 64 | 65 | 0.94 (0.4 - 2.2)
Asthma & Hayfever | 8 | 13 | 0.57 (0.2 - 1.6)
Tonsilitis | 17 | 17 | 1.0 (0.4 - 2.3)
Appendectomy | 0 | 4 | p=0.12
Pneumonia | 3 | 7 | 0.41 (0.1 - 1.9)
Gastroenteritis > twins | 2 | 5 | 0.69 (0.1 - 2.0)
Exposure to animals > twins | 16 | 3 | 6.42 (1.7 - 25) **

*p = 0.0007 **p = 0.003

The inaccuracy of retrospectively collected data is a concern in these studies. This method allowed an assessment of its validity by checking the agreement of the twins answers, this ranged between 75-99%.

Conclusion: These preliminary results confirms the increased frequency of episodes of "gastroenteritis" and reveals an increased exposure to animals. This method overcomes many of the traditional problems of case-control studies. The greater degree of matching increases the statistical power and comparing answers provides an internal validation of the data.

FAMILIAL AGGREGATION AND CONCORDANCE IN CLINICAL CHARACTERISTICS IN CROHN’S DISEASE. M PEETERS, H NEVENS, F BAERT, M HIELE, A-M DE MEYER, R VLEITNICK, P RUTGEERTS. Centre for Gastrointestinal Research, University of Leuven, B-3000 Belgium.

Background: Age adjusted risks are important for genetic counselling and modelling in Crohn’s disease. Moreover, knowledge of concordance in disease characteristics is important to define phenotypic subtypes.

Aims: First, to determine familial occurrence and age adjusted risks in first degree relatives of Crohn’s patients compared to controls. Second, to evaluate the agreement in disease characteristics within Crohn’s families.

Methods: Crohn’s patients (n=640) and controls (n=800) were questioned about familial occurrence of IBD in their first degree relatives. In addition agreement for age at diagnosis, initial disease location, disease behaviour and number of bowel resections was determined in 68 multiply-affected families and 100 unrelated Crohn’s patients.

Results: Crohn’s probands had a more frequent (p<0.001) positive family history for Crohn’s disease (13.6%) than controls (1.4%). Risk estimates were significantly higher in relatives of patients compared to controls. The highest age adjusted risk for IBD was found in offspring (10.4%). Especially daughters of Crohn’s patients showed an important IBD risk (12.6%). Parents were significantly (p<0.001) older at diagnosis than their offspring. Within generation age at diagnosis was very similar (r=0.69, p<0.001). The initial disease location showed a significant agreement within familial cases (κ=0.285; 0.057-0.512). Especially between siblings the agreement was striking (κ=0.372; 0.119-0.626).

Conclusions: Our large family study provides for the first time age adjusted risks for a European population. The clinical impression of familiality in disease characteristics was confirmed by using an objective measure of agreement, namely the κ-value.
SOCIAL CLASS, ETHNICITY & SMOKING IN A NATIONALLY REPRESENTATIVE COHORT WITH CROHN'S DISEASE.
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Introduction: There are few studies concerning the frequency of Crohn's disease (CD) among different ethnic groups within the UK. Likewise, there are few reports concerning the relevance or not of social class. Smoking has been shown to be a risk factor for CD. The fourth Morbidity and Mortality in General Practice (MMG4) represents a study of why patients in England or Wales visit their GP. It was conducted in 1991-92 and covered a population of 468,042. Data concerning ethnicity, social class, economic activity, smoking status (in the last week) and living in an urban or rural environment had been collected in MMG4.

Methods: We have previously identified 291 patients reported to have CD and in whom the diagnosis was not refuted subsequently. Controls were selected from the same practice as the case and matched for gender and age. Data were analysed in conditional logistic regression models using the GLIM4 program. Odds ratios (OR) for individual factors were obtained with 95% confidence intervals.

Results: 1682 controls were selected. The OR for Afro-caribbean's was 3.19 (95% confidence interval 2.86-3.53) and for Indians 1.03 (1.00-1.09). Compared with social classes 1 & 2 the OR for social class 3-non-manual was 1.21 (0.79-1.85), 3-manual 0.70 (0.48-1.30) and 4 1.08 (0.72-1.64). There was no significantly increased risk of being unemployed, OR 0.63 (0.29-1.36) but there was an increased risk of being registered for home help, OR 1.08 (1.02-1.17). There was no correlation between smoking and Crohn's disease (p>0.05).

Conclusion: This confirms that CD is more likely to occur in those who smoke and live in an urban environment. Social class is not a significant factor for CD. It may be more common in those of Afro-caribbean but not Indian origin.

T63
LOW APPENDICECTOMY RATES IN PATIENTS WITH ULCERATIVE COLITIS: EFFECT IS INDEPENDENT OF SMOKING.
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It has recently been noted that there is an inverse relationship between appendicectomy and ulcerative colitis (UC) in a Belgian study. This has not yet been confirmed in the U.K. There is also a possibility that this relationship may be linked with smoking. This case controlled, prospective study examines the frequency of appendicectomy and smoking in a stable U.K. population.

Methods: A total of 228 patients with a definitive diagnosis of inflammatory bowel disease were prospectively interviewed by a standardised questionnaire. There were 112 patients with a diagnosis of ulcerative colitis and 116 patients with Crohn's disease. 112 controls were recruited from accident and emergency department and were matched for age and sex.

Results: The appendicectomy rates amongst patients with UC was 2.7% (3/112) which was significantly lower compared to 16.1% (18/112) amongst controls (P<0.005, Odds ratio: 6.96, 95% C.I. 3.77-19.3). 2 patients with UC had their appendicectomy performed for diagnosis. Appendicectomy rates amongst patients with Crohn's disease was significantly greater than controls: 34% (18/112) vs. 16.1% (39/116), (P<0.01; OR: 2.65, 95% C.I. 1.51-4.14) but includes 9 patients who had appendicectomy as part of surgical intervention for Crohn's disease. The strong association of UC with non-smoking is confirmed (2% vs 42%, P<0.001, OR: 35.6, 95% C.I. 30.8-46.8). However there appears to be no association between smoking and appendicectomy in all three groups.

Conclusions: UC is associated with significantly lower appendicectomy rates than controls while appendicectomy rates in Crohn's disease is higher. This finding is independent of smoking. This intriguing finding may have implications in the aetiology of inflammatory bowel disease.

T64
THE ARTICULAR DISTRIBUTION AND CLINICAL COURSE OF THE PERIPHERAL ARTHROPATHIES IN ULCERATIVE COLITIS (UC).
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The association between UC and peripheral arthropathy is well recognised, but there are no diagnostic criteria and no information on their long term outcome. We have reviewed the records of patients with UC currently under review in Oxford and by subdividing them into 'reactive' (ReA), peripheral(PEA) and arthralgia have assessed the frequency and clinical course of the arthropathies.

The case notes were reviewed for the presence of arthritic symptoms and signs. Reactive arthropathies involved less than 5 joints involving a weightbearing joint with recorded evidence of joint swelling or effusion. Peripheral arthropathies involved 5 or more joints with evidence of swelling or effusion. Poorly defined joint syndromes and complaints without joint swelling were classified as arthralgia. Coincident rheumatic complaints, general features of UC and other extraintestinal manifestations were also recorded.

There were 860 patients under active review, of which 117 (13.6%) complained of peripheral joint problems 45 (5.0%) had arthralgia, 27 (3.3%) ReA and 22 (2.6%) PEA. In addition 6 patients had known rheumatoid arthritis and 39 had osteoarthritis. 23 of 27 patients (85.2%) with ReA had an episodic course of which 7 (30.4%) had more than one episode, and 4 (14.8%) had a continuous course. The most commonly reported joint was the ankle being affected in 23/27 cases with an effusion present in 10. Of 22 patients with peripheral arthritis 18 (82.8%) had a continuous course from 10-180 months, 2 (9.1%) were episodic, and 2 (9.1%) had only a single attack. The metacarpophalangeal joints were the most commonly involved (14/18) and there were no joint effusions recorded.

We have demonstrated two distinct forms of peripheral arthropathy in UC with different distributions and natural histories, ReA, which occurs as a single episode in 59.3% of cases and pursues a continuous course in only 14.8%, and PEA which pursues a continuous course in 82.8% of cases and only occurs as a single episode in 9.1%.
BONE DENSITY AND BONE TURNOVER MARKERS IN PATIENTS WITH CROHN’S DISEASE. R J Robinson, S J Igal, R P Whikeyer, F Al-Azzawi, I Ludwig, Gastrointestinal Research Unit, Leicester General Hospital, Gwendolen Road, Leicester.

Patients with Crohn’s disease (CD) are at risk of osteoporosis but the underlying pathophysiology is unclear. The aim of this cross-sectional study was to assess bone mineral density (BMD) in CD and use bone turnover markers to investigate the possible mechanisms of bone loss.

Methods: 12 patients with CD (32 premenopausal females, 48 males, mean age 38.3 (±13.2) years) were studied. Bone mineral density at the lumbar spine and total hip were measured, and serum markers of bone turnover (osteocalcin, deoxypyridinoline (DpYr), and procollagen type I-propeptide (PcIP)) measured. Urinary deoxyxypyridinoline (DpYr) was measured to assess bone resorption.

Results show that bone mineral density was lower than expected. The mean bone density of the lumbar spine was 0.84 (±0.24) SD below normal. Bone turnover markers were significantly increased in CD patients compared to controls. Mean PICP and DpYr were 98.1 (±47.6) and 10.2 (±6.7) nM/mM creatinine, respectively. Serum osteocalcin was increased to 2.25 (±1.02) nM/litre. No significant further differences in bone turnover markers were noted in patients with low bone density but DpYr was significantly correlated with body mass index (r=0.35; p<0.05). Urinary DpYr was not significantly correlated with any other markers but was positively correlated with BMD at Ward’s triangle (r=0.23, p<0.02). PICP was negatively correlated with BMD at the lumbar spine (r=-0.24, p<0.02) and trochanter (r=-0.22, p<0.03). Bone turnover markers were not significantly different in patients with and without Crohn’s disease.

Conclusions: The study confirms that low BMD occurs commonly in patients with CD, and a high proportion of patients have increased bone resorption as evidenced by raised DpYr. Our results suggest that a variety of mechanisms may operate to reduce BMD in patients with CD.

IMPRESSIVE HISTOLOGIC IMPROVEMENT AFTER TNF-ANTIBODY (cA2) THERAPY IN ACTIVE CROHN’S DISEASE. Baert F, Peeters M, D’Haens G, Geboes K*, Ectors N*, Rutgeerts P. Dept of Gastroenterology and Pathology, University of Leuven, Belgium.

TNF-α is an important mediator of inflammation in Crohn’s disease (CD). We studied the histologic effects of cA2 therapy in a systematic way. Biopsies of 13 patients with active CD before and after 4 weeks of therapy were reviewed. Grade 2 or 3 inflammatory changes were present in 10 patients (80%). The mean number of inflammatory changes was 2.9±1.9 before and 0.6±0.6 after therapy (p<0.001).

Results: The mean total activity score in the cA2 treated group dropped from 6.7 (2.12) to 3.0 (0.7) in ileitis and from 7.6 (2.12) to 3.0 (0.8) in colitis compared to 11 (10-12) before and 9 (6-12) after placebo therapy.

Conclusion: Anomalonc TNF-α antibody therapy significantly improves histologic disease activity in active Crohn’s ileitis and colitis. The improvement is mainly due to a dramatic decrease of the inflammatory infiltrate parallel to a reduction of HLA-DR expression and the number of CD68+ and LFA1+ cells.
**GUT LUMINAL NEUTROPHIL MIGRATION DEPENDS ON THE ANATOMICAL SITE OF CROHN’S DISEASE**

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Background: Gut luminal neutrophils can be studied by using whole gut lavage fluid (WLF) obtained after bowel cleansing by a polyethylene glycol-electrolyte solution (Klean-Prep, Norgine). Cytology or an enzyme-substrate reaction to detect granulocyte elastase (GE) can be performed on centrifuged WLF. The chemotactants responsible for luminal neutrophil migration are unknown, but bacterial products are potential candidates.

Methods: 70 patients with well characterised active CD underwent whole gut lavage. The clear fluid obtained after complete gut cleansing was stored at -70°C. GE was assessed using the specific chromogenic substrate L-Prolylglutamyl-L-prolyl-L-valine-p-nitroanilide (Quadratech, Epsom). The lower limit of detection by this assay was 2.7 nkat/l. Patients were divided in 6 groups according to the distribution of macroscopic disease.

Results: 21 of 31 patients with Crohn’s colitis had detectable GE, (median 238, range <38-274 nkat/l), whereas only 1 out of 10 patients with small bowel involvement had detectable GE (median <36, range <38-215 nkat/l; P<0.001). 10 out of 13 patients with both small bowel and colonic disease had detectable GE (median 180, range <38-170 nkat/l; P<0.02 vs small bowel). In the 6 patients with recurrent small bowel disease who had prior ileocolic resection, 5 had detectable GE, and the concentrations were significantly higher (median 228, range <38-1240 nkat/l) than in those with small bowel disease but no resections (P<0.05). 6 patients with an anastomosis and patients with postoperative disease alone had undetectable GE. None of the 23 patients with Crohn’s colitis had received long-term corticosteroids or therapy for possible tropical sprue before his diagnosis was established - he had no detectable GE in lavage fluid. A high GE concentration of 548 nkat/l, was detected in a patient with small bowel CD, who had received high dose long-term NSAsids for relapsing spondyloysis.

Conclusion: Neutrophil migration into the lumen of the gut is a feature of colonic but not small bowel Crohn's disease suggesting that bacterial flora-derived neutrophil chemotactants may play a role in gut neutrophil migration. This hypothesis is further supported by the high GE concentrations found in recurrent small bowel disease after ileocolic resection.

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**GRAFT INFILTRATING LYMPHOCYTES IN LONG TERM LIVER TRANSPLANTS. A MARKER OF TOLERANCE?**

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Clinical tolerance is present in a significant proportion of long term liver transplant recipients, although to date it has not been possible to reliably identify these recipients by conventional clinical, or biochemical, parameters. The present study examined whether immunohistochemical analysis of pre-weaning liver biopsies could discriminate unique patterns in patients subsequently found to be tolerant.

Methods: 37 biopsies: 27 from patients with stable graft function 5 years, or more post transplant, and 10 from patients experiencing acute rejection were studied. Anti-CD3, CD4, CD8, CD45RO, CD56, and CD68 staining was performed.

Results: Of the 27 long term patients, 20 subsequently underwent weaning of their immunosuppression; 6 were tolerant, 5 achieved substantial reductions in their immunosuppression (partially tolerant), and 9 were unable to achieve any substantial reduction (intolerant). Reduced numbers of lobular CD8+ and CD3+ cells were present in the tolerant as compared to the intolerant grafts (median 15 vs 22 P<0.01, 15 vs 26 cells/high powered field [hpf] respectively, P<0.03). Similarly, there were fewer numbers of lobular CD3+, CD8+, CD45RO+ cells in tolerant grafts when compared with acute rejection (16 vs 32 P<0.01, 15 vs 31 P<0.01, 17 vs 24 P<0.03 median cells/hpf respectively). Unexpectedly more lobular CD45RO+ were present in tolerant when compared with partially tolerant patients (17 vs 10 mean cells/hpf, P<0.05).

Conclusions: In all long term liver grafts, infiltrating lymphocytes are present, and with these grafts from patients intolerant of immunosuppression withdrawal they are phenotypically similar to those during acute cellular rejection. Fewer CD8+ positive cells correlate with graft tolerance, and may aid in the identification of patients in whom successful withdrawal of immunosuppression may be undertaken.

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**TWO-STAGE GENOME-WIDE SEARCH IN INFLAMMATORY BOWEL DISEASE: STRONG EVIDENCE FOR SUSCEPTIBILITY LOCUS ON CHROMOSOMES 3, 7 AND 12**

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Concordance rates in siblings and twin pairs have provided strong evidence that genetic predisposition is important in the pathogenesis of the inflammatory bowel diseases (IBD). However, neither Crohn’s disease (CD) nor ulcerative colitis (UC) has a simple Mendelian pattern of inheritance; the model which seems most pertinent to inflammatory bowel disease is that of a heterogeneous group of polygenic disorders. In the present study, a systematic two-stage search of the human genome for susceptibility genes in inflammatory bowel disease was performed, involving 186 affected sibling pairs.

In the first stage, 89 sibling pairs with IBD were genotyped at 260 microsatellite markers spanning the 22 autosomes, using fluorescence labelled primers for polymerase chain reaction, and semi-automated DNA fragment sizing technology. Allele sharing in affected sibling pairs provided evidence for linkage with 12 markers in 5 distinct regions of chromosomes 2, 3, 7, 12 and 15 (p<0.001 for an individual marker, or adjacent markers each with p<0.01).

In the second stage, a further 97 affected sibling pairs with IBD were genotyped; linkage for the clustered markers on chromosomes 3, 7 and 12 was confirmed.

Combining data from the first and second stages provided striking evidence for linkage with 3 adjacent markers on chromosome 3, D2S2159 (p = 2.6 x 10^-5), D2S3569 (p = 5.7 x 10^-10), and D2S5242 (p = 2.1 x 10^-10), lod score 4.845, 4.29 and 6.29, respectively. Three markers on chromosome 7, D7S409 (p = 1.3 x 10^-4), D7S401 (p = 2.6 x 10^-5), and D7S343 (p = 2.9 x 10^-4), lod score 1.29, 3.29 and 7.59, respectively.

Three markers on chromosome 12, D12S342 (p = 2.0 x 10^-10), D12S349 (p = 2.8 x 10^-10), and D12S350 (p = 2.8 x 10^-10), lod score 2.04, 2.99 and 2.34, respectively.

These data provide compelling evidence for the chromosomal location of IBD susceptibility genes. Strong candidate genes are present in each of the regions which have been implicated, and are currently being studied.

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**MONITORING OF LIVER OXYGENATION BY NEAR INFRARED SPECTROSCOPY.**

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Adequate oxygenation of the liver is fundamental to the outcome of liver surgery and orthotopic liver transplantation (OLT). Near-Infra-Red Spectroscopy (NIRS) is a novel technique which can non invasively measure tissue oxygen (HbO2), deoxyHb and total haemoglobin (THb). The results of NIRS on the liver have not previously been compared with blood flow.

Large Landrace pigs (n=7) underwent laparotomy under general anaesthesia. Electromagnetic flowmeters were placed around the portal vein and hepatic artery and the NIRS probes on the surface of the right lobe. NIRS and flow were recorded continuously. Baseline readings were taken on achieving a steady state and subsequent recordings following occlusion of the hepatic artery (HA), portal vein (PV) and both (HA+PV). The correlation between NIRS and hepatic blood flow is shown.

![Table showing oxygenation parameters before and after interventions](https://via.placeholder.com/150)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>HA clamp</th>
<th>PV clamp</th>
<th>HA &amp; PV clamp</th>
</tr>
</thead>
<tbody>
<tr>
<td>HbO2 (μmol/l)</td>
<td>-27 ± 20</td>
<td>-185 ± 104</td>
<td>-265 ± 92</td>
</tr>
<tr>
<td>Deoxy Hb (μmol/l)</td>
<td>-6 ± 26</td>
<td>-38 ± 48</td>
<td>-54 ± 40</td>
</tr>
<tr>
<td>HA (mM/l)</td>
<td>0</td>
<td>178 ± 128</td>
<td>0</td>
</tr>
<tr>
<td>PV (mM/l)</td>
<td>817 ± 191</td>
<td>0</td>
<td>0</td>
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</tbody>
</table>

Changes relative to baseline. *values are in means±SD.

The baseline HA and PV blood flow were 148±110 and 741±170 mMin. THb showed a strong correlation with total hepatic blood flow (r=0.932, P<0.001).

We would conclude from this study that NIRS would be an accurate non invasive method of assessing organ perfusion and oxygenation in patients undergoing liver resection or transplantation.