J. MICHAEL HENDERSON, MD, FRCS;
SHERIF S. HANNA, MD, FRCS[C]

Effective Liver Blood Flow: Determination by Galactose Clearance

Effective liver blood flow is the portion of total flow that perfuses functional sinusoids and is available for metabolic exchange. Clearance of galactose from blood at concentrations below 10 mg/dl (0.555 mmol/l) measures this index and is calculated during continuous infusion of 5% D-galactose at a rate of 50 mg/min. The low galactose concentrations are measured accurately by a new fluorometric assay, which gives a precision ±0.2 mg/dl (0.011 mmol/l). In healthy people, plasma galactose clearance was 1366 ± 172 ml/min, and hepatic extraction was 95%. Clearance in cirrhotics depends on the stage of their disease: in a stable group of patients with advanced cirrhosis, clearance was 835 ± 87 ml/min with hepatic extraction ranging from 60% to 95%. The day-to-day coefficient of variation was 4.5%. Direct comparison with flow-probe liver blood flow measured in 11 normal dogs showed that galactose clearance was not significantly different. These findings support the hypothesis that galactose clearance correlates with effective liver blood flow.

Le débit sanguin hépatique utile est la partie du débit total qui perfuse les capillaires sinusoïdes fonctionnels et qui participe aux échanges métaboliques. La clairance du galactose à des concentrations inférieures à 10 mg/dl (0.555 mmol/l) permet de mesurer cet index. Elle est calculée durant une perfusion continue de D-galactose à 5%, à la vitesse de 50 mg/min. Les faibles concentrations de galactose sont mesurées avec exactitude grâce à une nouvelle méthode de dosage fluorimétrique qui donne une précision de ±0.2 mg/dl (0.011 mmol/l). Chez des sujets sains, la clairance du galactose plasmatique était de 1366 ± 172 ml/min, et l'extraction hépatique de 95%. Chez l'individu cirrhotique, la clairance dépend du stade d'évolution de la maladie: chez un groupe de patients stables souffrant de cirrhose avancée, la clairance était de 835 ± 87 ml/min avec une extraction hépatique variant entre 60% et 95%. Le coefficient de variation quotidien était de 4.5%. La comparaison directe avec le débit sanguin hépatique mesuré par cathétérisme chez 11 chiens normaux, a montré que la clairance de galactose n'était pas significativement différente. Ces résultats corroborent l'hypothèse voulant que la clairance du galactose soit en rapport avec le débit sanguin hépatique utile.

Homer Smith and associates,[1] in their classic work on renal clearance in 1938, defined effective renal blood flow as the blood flow to active excretory tissue. Effective flow can only be measured by a clearance technique, and they showed that the best substance (a) had to be that with the highest possible clearance, (b) must be used at sufficiently low blood concentrations to be below the maximal removal capacity of its pathway and (c) must not be interfered with by other solutes. Of the many substances tested, Hippuran was found most suitable and has been widely used to measure effective renal blood flow.

Can the same principles be applied to measure effective liver blood flow? There are obvious differences between the liver and kidney, such as the dual blood inflow to the liver and the inability to collect test substances cleared by the liver. However, by the application of basic pharmacokinetics, blood clearance can be calculated for substances highly extracted by the liver. Clearances will approximate effective liver blood flow if two conditions are fulfilled. First, the test substance must be totally cleared at each pass through functioning liver tissue, and second, it must be removed by the liver alone. Effective liver blood flow is that portion of total flow which is available for metabolic exchange.

Galactose was chosen as a potential test substance fulfilling these criteria because previous work has shown that there is a high clearance of the substance from the liver, particularly at low blood concentrations.[2,3] This ability of the liver to clear galactose from blood stems from several facts: (a) galactose is actively transported into the hepatocyte, (b) it has its own metabolic pathway, converting it to glucose, (c) it is water soluble and (d) it has no protein-binding ability. Clearance of galactose from blood can be studied as both zero-order and first-order kinetic phases. At blood concentrations above 40 mg/dl (2.22 mmol/l) the metabolic pathway is saturated and the liver removes a maximum amount of galactose, depending on the functional hepatocyte mass. This observation has been substantiated and used by many as a true test of liver function.[4,5] In contrast, galactose clearance from blood at concentrations below 40 mg/dl (2.22 mmol/l) shows an exponential decline, the pathway is no longer saturated and the liver blood flow is rate-limiting in clearance.

This paper reviews the development of a method for measuring galactose clearance at blood concentrations below 10 ml/dl (0.555 mmol/l). We present data in support of our hypothesis that this method fulfils the criteria of a test substance suitable to measure effective liver blood flow. The several stages of development will be reviewed in turn, with emphasis on methodology and clinical relevance. The stages are: (a) a new biochemical method with the requisite sensitivity for measuring galactose clearance at blood concentrations below 10 mg/dl (0.555 mmol/l), (b) study of the clearance kinetics of galactose in this concentration range to determine if galactose approaches the ideal test substance for measuring effective liver blood flow and (c) validation of galactose clearance against flow-probe measurements in nor-

From the departments of surgery, Emory University School of Medicine, Atlanta, Ga. and Sunnybrook Medical Centre, University of Toronto, Toronto, Ont.

Presented at the 5th annual meeting of the Canadian Association of General Surgeons, Quebec City, PQ, Sept. 16, 1982

Supported by US Public Health Services research grant no. AM-15736 and Sunnybrook Medical Centre research grants nos. 5390 and 5430

Accepted for publication Nov. 1, 1982

Reprint requests to: Dr. S.S. Hanna, Consultant general surgeon, Sunnybrook Medical Centre, 2075 Bayview Ave., Toronto, Ont. M4N 3M5

mal dogs, conditions under which effective flow should approximate anatomic flow.

Assay of Low Galactose Concentrations in Blood

The biochemical requirement to enable calculation of clearance to within ±5% was the ability to assay galactose in the blood concentration range 0 to 10 mg/dl (0 to 0.555 mmol/l) with an accuracy of ±0.2 mg/dl (0.011 mmol/l). This projected requirement was calculated from the pathophysiologic limitations of liver blood flow. The details of the new method have been published elsewhere,[6] but we shall emphasize some salient features.

Work on galactosemia showed the feasibility of measuring relatively high galactose concentrations in a drop (25 μl) of blood. The converse, however, proved more difficult; at low galactose concentrations, interfering compounds in blood came into play. The main interfering substances, uric acid and glutathione, are coprecipitated with protein by zinc/alkali in the initial assay step. The clear filtrate so formed is then assayed for galactose by a continuous flow method. The primary reaction is the oxidation of galactose by galactose oxidase, with subsequent detection of hydrogen peroxide by peroxidase and a fluorometric substrate. Pretreatment of samples for removal of interfering substances, use of fluorometry and of p-hydroxphenylacetic acid as the fluorometric substrate and optimizing the concentration of galactose oxidase were the main developmental improvements in this assay. Its precision, in 20 samples assayed in duplicate at 1, 4, 7 and 10 mg/dl (0.056, 0.222, 0.389 and 0.555 mmol/l), four times on each of 5 days, is shown in Table I. This represents an accuracy of ±0.2 mg/dl (0.011 mmol/l), which is in the necessary range for the proposed clearance studies.

At these low concentrations certain technical pitfalls can reduce this accuracy and need to be avoided:

- Sample collection (3 ml) should be into fluoride (Vacutainer tube M3273 PS; Becton Dickinson Co., Rutherford, NJ) and samples should be placed on ice. Galactose is metabolized by erythrocytes at an hourly rate of 1.4 mg/dl (0.078 mmol/l) at 37°C;[7] this loss will substantially alter sample concentrations but is abolished by using fluoride collection. In terms of total blood clearance this erythrocyte metabolism contributes only 2.3%.
- For sample storage, plasma must be separated within 1 hour of collection and stored at −20°C.
- Whole blood galactose concentrations can also be assayed. Pretreatment of samples by zinc/alkali hemolyses the erythrocytes and precipitates all interfering compounds, rendering a clear filtrate for analysis. For those samples stored as plasma for subsequent analysis, the conversion of plasma to whole blood concentration was calculated by concomitant assay in 10 samples, the conversion factor being

$$1 - \frac{(0.5 \times \text{hematocrit})}{100}$$

- To ensure stability of galactose, whole blood assay should be done on fresh samples. The zinc/alkali filtrate should be assayed within 3 hours. In plasma stored at −20°C no galactose is lost at 2 months.
- Galactose oxidase of first-class purity is required for the low galactose concentrations under study. We have found Worthington Chemical Co. (Freehold, NJ) to be the most reliable supplier; five other sources have been checked and the only other supplier with sufficiently pure enzyme was P.L. Biochemicals (Milwaukee, Wisc.). The fluorometric substance p-hydroxphenylacetic acid is less stable than the other reagents; stock solution should not be carried for more than 3 weeks.

In summary this new assay has the requisite sensitivity for calculating galactose clearance in the 0 to 10 mg/dl (0 to 0.555 mmol/l) blood concentration range, but attention to detail is required to maintain this accuracy.

Clearance Kinetics of Galactose at Concentrations below 10 mg/dl (0.555 mmol/l)

This series quantitates the distribution and elimination kinetics of galactose during continuous intravenous infusion of 20 to 100 mg/min of galactose. Continuous infusion to steady state proved to be the best method for clearance data analysis in this concentration range. All the clearance values in this series are plasma values. The details are published elsewhere.[7]

Distribution and Elimination

Fig. 1 illustrates the kinetics of distribution and elimination in a healthy cirrhotic subject infused with 5% D-galactose at 50 mg/min for 140 minutes and monitored for 60 minutes thereafter.

In a series of five healthy subjects and five cirrhotic patients thus studied, the data were analysed by a curve-fitting program with the following results.

- The infusion and postinfusion curves, computer-fitted to multicompartmental models, best fitted a single compartment model.
- All subjects achieved 95% of steady state by 60 to 80 minutes.

Table I—Galactose Assay. Precision and Reproducibility Studies at Four Concentrations Measured Four Times in Each Run on 5 Days

Precision/reproducibility	Expected concentrations, mg/dl			
	1	4	7	10
Overall mean (± SEM)	0.97 (±0.066)	3.97 (±0.060)	7.07 (±0.090)	9.85 (±0.051)
Standard deviation				
Sample to sample	0.074	0.151	0.087	0.130
Within run	0.083	0.151	0.154	0.207
Day to day	0.166	0.198	0.243	0.217

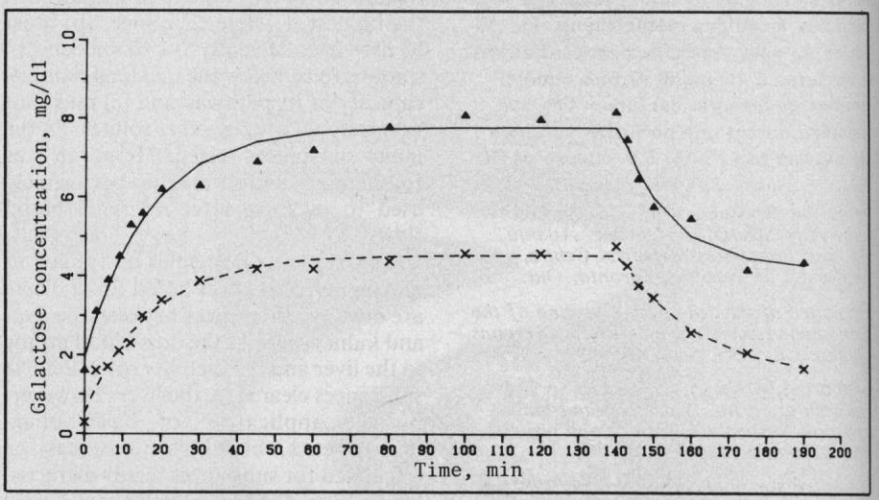

FIG. 1—Plasma galactose concentration versus time curve during infusion of 50 mg/min of galactose for 140 minutes and for 60 minutes postinfusion, in healthy patient and cirrhotic patient. Triangles (cirrhotic patient) and crosses (healthy patient) indicate measured values. Solid line (cirrhotic) and broken line (healthy) indicate predicted values.

• Clearance can be calculated from either of the following equations: (a) clearance_{ss} = infusion rate/($C_{ss} - C_o$) or (b) clearance_{AUC} = total dose infused/$AUC_{0-\infty}$, where C_{ss} = estimated plasma steady state concentration, C_o = plasma concentration at time zero (range 0.1 to 1.9 mg/dl [0.006 to 0.105 mmol/l]) and $AUC_{0-\infty}$ = area under plasma concentration versus time curve from zero time to infinity.

The mean (± SD) clearances_{ss} for the healthy subjects (1366 ± 172 ml/min) and cirrhotics (835 ± 87 ml/min) were not significantly different from the mean clearances_{AUC} (1396 ± 481 and 824 ± 140 ml/min respectively).

In all further studies clearance was calculated from equation a. Estimated plasma steady state concentration was attained more rapidly by a priming bolus injection of 500 mg galactose at zero time and was defined from five blood samples drawn from 60 to 100 minutes.

Galactose Infusion at Two Rates

In five normal subjects, infused at rates of 50 and 100 mg/min, and nine cirrhotics, infused at 25 and 50 mg/min, we tested the hypothesis that clearance is a first-order kinetic reaction and is independent of hepatic extraction. For this to be true, doubling the infusion rate should double $C_{ss} - C_o$, or clearance should be unchanged. The ideal was approached in all subjects: the ratio of clearance_2 to clearance_1 was 0.919 ± 0.088; this equates to a mean 8.1% reduction in clearance at twice the infusion rate. Several factors, such as change in baseline, extrahepatic removal and decreased hepatic extraction, may contribute to this.[7]

Hepatic Extraction

Extraction across functioning liver tissue needs to be calculated to validate the fact that clearance measures effective flow; this extraction cannot be measured. But how much total hepatic extraction is achieved at these low galactose concentrations?

In two healthy subjects hepatic extractions were 93% and 95%. In nine patients with cirrhosis extraction ranged from 60% to 95% (mean 79%). These data are important in showing that galactose is highly extracted, even by the cirrhotic liver, and support the concept that from 60% to 95% of flow in the cirrhotics was effective flow.

Day-to-Day Reproducibility

For any test to be of value in following the progression of disease or the follow-up of patients, it must be reproducible. Assessment of this variable in 11 subjects, studied twice within 1 week under identical conditions, showed that the coefficient of variation was 4.5%.

Extrahepatic Clearance

The ideal test substance for measuring effective liver blood flow must be cleared by the liver alone. We have already shown that 2.3% of galactose clearance at these blood concentrations can, at least in theory, be metabolized by erythrocytes. Renal galactose clearance, calculated in six subjects with a timed urine collection during galactose infusion at plasma steady state, amounted to 2% of total clearance. Galactose is a ubiquitous compound; cell-wall synthesis requires galactose and the body continually turns over a small background pool. It is possible there are other extraheptic clearance routes; however, the available data suggest that by far the greatest clearance is by the liver.

Conclusions

These kinetic studies on the clearance of galactose at concentrations of 0 to 10 mg/dl (0 to 0.555 mmol/l) show that it approaches the ideal test substance for measuring effective liver blood flow: (a) it is kinetically simple to analyse at steady state during continuous infusion, (b) it is avidly removed by the liver, with minimal extrahepatic clearance and (c) there is indirect evidence supporting virtually complete extraction by functional liver tissue on each pass.

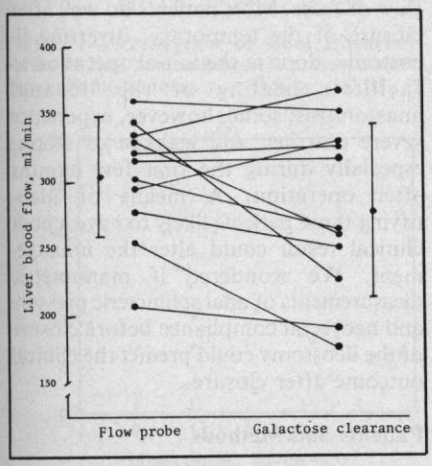

FIG. 2—Galactose clearance and flow-probe measurement of liver blood flow in 11 healthy dogs. Analysis of variance shows no significant difference between these two methods.

Galactose Clearance and Flow-Probe Measurement of Liver Blood Flow in Healthy Dogs

One of the steps in validating any new method must be direct comparison with the accepted standard. There is no accepted standard for measuring effective liver blood flow, but assuming that effective flow will approximate total flow in the normal liver, we made this comparison in normal dogs using intraoperative flow-probe measurement for anatomic flow.

Eleven conditioned dogs, with a mean (± SD) weight of 10.2 ± 0.79 kg were studied by the two methods concurrently. Galactose clearance was measured during continuous infusion of 10 mg/min of 5% galactose. Plasma steady state was defined over 50 minutes when the dog was hemodynamically stable. This was corrected to blood C_{ss} as previously defined and clearance calculated from equation a. Flow-probe measurement of anatomic liver blood flow was made by surgical dissection and isolation of the hepatic artery and portal vein. Flow probes were placed around each vessel and, with the dog stabilized, two recordings were made during definition of the galactose steady state. Two measurements were compared by analysis of variance (Fig. 2).

The mean galactose clearance intraoperatively (279 ± 64 ml/min) was not significantly different from the mean flow-probe measurement (304 ± 44 ml/min). The coefficient of variation between the two methods was 12.5%.[8]

The flow-probe method is the most accurate one available for measuring liver blood flow, but is is not clinically applicable. These data support the hypothesis that galactose clearance measures effective liver blood flow, which, in this particular model, approximates anatomic flow.

Comment

Normal liver function depends on hepatocellular integrity and adequate liver perfusion. Quantitative measurement of both these indices gives a better profile of how the liver is responding to a disease process than do standard liver function tests, such as measurement of serum bilirubin, glutamic oxaloacetic transaminase and albumin levels and the prothrombin time. Galactose clearance, as presented in this paper, quantitates the flow component of function.

The removal of substances from blood by hepatic clearance is influenced by three factors: the intrinsic elimination capacity (hepatocyte function), hepatic extraction and liver blood flow. Galactose clearance, in the blood concentration range 0 to 10 mg/dl (0 to 0.555 mmol/l), measures clearance at infusion rates one fifth to one tenth of intrinsic elimination capacity, is virtually independent of hepatic extraction and is thus a flow-dependent clearance.

The liver has a myriad of metabolic pathways; to develop a full functional profile, other pathways that determine hepatocellular integrity as well as liver

perfusion must be studied in a similar manner. Hepatocyte function can be assessed quantitatively by measuring the clearance of test substances in which intrinsic elimination capacity is rate-limiting (e.g., methionine).[9] Alternative substances, those with a high first-pass extraction rate, in which liver blood flow is rate-limiting should be developed to measure flow-dependent clearance.[10]

The concept of quantitative assessment of liver function is receiving increased interest. We believe that by developing a profile of function and flow-dependent clearance measurements a better understanding and quantitation of disease states will be achieved.

References

1. SMITH HW, GOLDRING W, CHASIS H: The measurement of the tubular excretory mass, effective blood flow and filtration rate in the normal human kidney. *J Clin Invest* 1938; 17: 263–78
2. TYGSTRUP N, WINKLER K: Galactose blood clearance as a measure of hepatic blood flow. *Clin Sci* 1958; 17: 1–9
3. WALDSTEIN SS, ARCILLA RA: Measurement of hepatic blood flow by clearance methods; a review and a theoretical basis for a new noncatheterization method. *Am J Dig Dis* 1958; 3: 137–58
4. TYGSTRUP N: The galactose elimination capacity in control subjects and in patients with cirrhosis of the liver. *Acta Med Scand* 1964; 175: 281–9
5. COLCHER H, PATEK AJ JR, KENDALL FE: Galactose disappearance from the blood stream; calculation of a galactose removal constant and its application as a test for liver function. *J Clin Invest* 1946; 25: 768–75
6. HENDERSON JM, FALES FW: Continuous-flow fluorometry of low galactose concentrations in blood or plasma. *Clin Chem* 1980; 26: 282–5
7. HENDERSON JM, KUTNER MH, BAIN RP: First-order clearance of plasma galactose: the effect of liver disease. *Gastroenterology* 1982; 83: 1090–6
8. HANNA SS, HENDERSON JM: Liver blood flow in dogs measured by galactose clearance: reproducibility, probe comparison, and effect of portacaval shunt. *Surg Forum* 1982; 33: 178–9
9. HOROWITZ JH, RYPINS EB, HENDERSON JM, HEYMSFIELD SB, MOFFITT SD, BAIN RP, CHAWLA RK, BLEIER JC, RUDMAN D: Evidence for impairment of transsulfuration pathway in cirrhosis. *Gastroenterology* 1981; 81: 668–75
10. WILKINSON GR: Pharmacokinetics of drug disposition: hemodynamic considerations. *Annu Rev Pharmacol* 1975; 15: 11–27

JACQUES HEPPELL, MD; BRIAN M. TAYLOR, MD;
ROBERT W. BEART, JR., MD; ROGER R. DOZOIS, MD;
KEITH A. KELLY, MD

Predicting Outcome after Endorectal Ileoanal Anastomosis

The aim of this study was to determine whether anorectal manometry could predict clinical outcome after closure of a diverting ileostomy made at the time of colectomy, mucosal proctectomy and endorectal ileoanal anastomosis for chronic ulcerative colitis. Twenty-two patients were studied, 6 with ileoanal anastomosis and 16 with ileal pouch–anal anastomosis. Resting anal sphincter pressures and pressure-volume curves (compliance) of the neorectum were assessed before the diverting ileostomy was closed and results were correlated with frequency and leakage of stools 1 month after closure. Before stomal closure, the mean (± standard error of the mean) resting sphincteric pressure was 44 ± 5 cm H_2O, while neorectal compliance was 2.3 ± 0.3 ml/cm H_2O. One month later daily stool frequency was 12 ± 1, while severe leakage occurred in four patients during the day and in eight at night. The greater the sphincteric pressure and the compliance, the fewer were the number of stools per day ($p < 0.01$). When pressure and compliance were considered together as an index of anorectal function, the correlation of stool frequency and leakage was even stronger ($p < 0.001$). The authors conclude that anorectal manometry can predict early clinical outcome after ileoanal anastomosis.

Le but de cette étude était de déterminer si la manométrie ano-rectale pouvait prédire les résultats cliniques après fermeture d'une iléostomie de dérivation pratiquée au moment d'une colectomie, d'une protectomie de la muqueuse et d'une anastomose iléo-anale endorectale pour colite ulcéreuse chronique. Vingt-deux patients ont été étudiés, 6 porteurs d'une anastomose iléo-anale et 16 d'une anastomose entre le diverticule iléal et l'anus. Les pressions du sphinctère anal au repos et les courbes de pressions-volumes (compliance) du néorectum ont été mesurées avant la fermeture de l'iléostomie de dérivation et les résultats ont été reliés à la fréquence et à la fuite des selles 1 mois après la fermeture. Avant fermeture de l'abouchement, la pression sphinctérienne moyenne au repos (± l'erreur type) était de 44 ± 5 cm H_2O, alors que la compliance néorectale était de 2.3 ± 0.3 ml/cm H_2O. Un mois plus tard, la fréquence quotidienne des selles était de 12 ± 1, alors qu'une fuite importante est survenue chez quatre patients durant le jour et chez huit patients durant la nuit. Plus grandes étaient la pression sphinctérienne et la compliance, moins nombreuses étaient les selles quotidiennes ($p < 0.01$). Quand la pression et la compliance ont été pris ensemble comme indice de la fonction ano-rectale, une corrélation encore plus forte avec la fréquence des selles et les fuites a été observée ($p < 0.001$). Les auteurs concluent que la manométrie ano-rectale peut prédire le résultat clinique immédiat après anastomose iléo-anale.

From the gastroenterology unit and the department of surgery, Mayo Clinic and Mayo Foundation, Rochester, Minn., USA

Presented at the 5th annual meeting of the Canadian Association of General Surgeons, Quebec City, PQ, Sept. 17, 1982

Supported in part by the Medical Research Council of Canada, US Public Health Service and National Institutes of Health grants, nos. AM-18278 and RR-585, and the Mayo Foundation

Accepted for publication Nov. 1, 1982

Reprint requests to: Dr. Keith A. Kelly, Department of surgery, Mayo Clinic, Rochester, MN 55905

Colectomy, mucosal proctectomy and endorectal ileoanal anastomosis is an attractive alternative to proctocolectomy and conventional or continent ileostomy for patients with ulcerative colitis or familial polyposis.[1-4] The operation avoids a permanent stoma and preserves the transanal flow of feces. Most patients do well after closure of the temporary, diverting ileostomy, done at the initial operation to facilitate healing of the ileoanal anastomosis; some, however, experience severe diarrhea and leakage of stools, especially during the first few months after operation. A means of identifying those patients likely to have a poor clinical result could alter the management. We wondered if manometric measurements of anal sphincteric pressure and neorectal compliance before closure of the ileostomy could predict the clinical outcome after closure.

Patients and Methods

Twenty-two patients, 11 women and 11 men whose mean age (± standard error) was 31 ± 2 years (range from 16 to 48