

## CORRESPONDENCE

### PIVKA-II concentrations in patients with cystic fibrosis

Montalembert *et al* report that PIVKA-II was detected in 33% of patients with cystic fibrosis, while vitamin K<sub>1</sub> plasma concentrations were normal.<sup>1</sup> It is astonishing that despite daily supplements of 5-10 mg vitamin K<sub>1</sub>, PIVKA-II was detectable in these patients. The authors conclude that PIVKA-II is not associated with vitamin K deficiency, but with the use of antibiotics.

There is some doubt as to whether the assay for PIVKA-II used by the authors is reliable. Widdershoven *et al* compared different methods for measuring PIVKA-II and reported that techniques involving adsorption of normal factor II may result in false positive values, because the carboxylated prothrombin may not be removed completely.<sup>2</sup> Determination of PIVKA-II by monoclonal antibody was found to be the most specific and sensitive method.<sup>2</sup> We did not detect PIVKA-II in any of eight patients with cystic fibrosis who were supplemented with vitamin K<sub>1</sub> (4-30 mg/day).<sup>3</sup> In only one out of 16 unsupplemented patients with cystic fibrosis was PIVKA-II found (0.16 AU/ml). This patient took antibiotics, had a low vitamin K<sub>1</sub> concentration of 0.06 µg/l and a Thrombotest of 56%.<sup>3</sup>

The authors do not mention vitamin K<sub>2</sub>. Except for vitamin K<sub>1</sub>, vitamin K<sub>2</sub> must be accounted for when assessing vitamin K status. Antibiotics may disturb vitamin K<sub>2</sub> production by intestinal flora and hence reduce the amount of total vitamin K available for the carboxylation of PIVKA-II to functional factor II. A correlation between subnormal coagulation tests and antibiotics in cystic fibrosis was reported by Komp and Selden.<sup>4</sup> As there was no information on concentrations of vitamin K<sub>2</sub> it is impossible to establish normal values for vitamin K<sub>1</sub>. PIVKA-II, however, is a direct reflection of the availability of total vitamin K in the liver and hence is associated with vitamin K deficiency. In our study PIVKA-II was found in only one unsupplemented patient with cystic fibrosis, and hence we conclude that vitamin K deficiency occurs infrequently in cystic fibrosis.<sup>3</sup>

EAM CORNELISSEN  
AF VAN LIEBURG  
CG VAN OOSTROM  
LAH MONNENS

Department of Paediatrics,  
University Hospital Nijmegen,  
PO Box 9101, NL-6500 HB Nijmegen,  
The Netherlands

- 1 Montalembert M de, Lenoir G, Sant-Raymond A, Rey J, Lefrère JJ. Increased PIVKA-II concentrations in patients with cystic fibrosis. *J Clin Pathol* 1992;45:180-1.
- 2 Widdershoven J, Munster P van, De Abreu R, *et al*. Four methods compared for measuring des-carboxy-prothrombin (PIVKA-II). *Clin Chem* 1987;33:2074-8.
- 3 Cornelissen EAM, Lieburg AF van, Motohara K, Oostrom CG van. Vitamin K status in cystic fibrosis. *Acta Paediatr Scand* (in press).
- 4 Komp DM, Selden RF. Coagulation abnormalities in cystic fibrosis. *Chest* 1970;58:501-3.

Dr Lefrère *et al* comment:

Our PIVKA-II assay, based on the activity of staphylocoagulase, is widely used in many laboratories. The results obtained with this

procedure with those of laboratories measuring PIVKA-II with monoclonal antibody, in particular in patients with hepatocellular carcinoma.<sup>1,2</sup> Furthermore, we measured PIVKA-II concentrations in a large population of healthy individuals (blood donors) and obtained no false positive result in these individuals.

Dr Cornelissen *et al* do not raise the possibility of increased PIVKA-II in contexts other than vitamin K deficiency, such as hepatocellular carcinoma,<sup>1,2</sup> hepatoblastoma,<sup>3</sup> effect of oral anticoagulants<sup>4</sup> and cephalosporins.<sup>5</sup> Indeed, vitamin K deficiency is not the only mechanism to generate PIVKA-II. In hepatocellular carcinoma increased PIVKA-II concentration is probably due to an acquired enzymatic anomaly which disturbs the  $\gamma$ -carboxylation of all vitamin K dependent factors.<sup>6</sup> We could not explain this increase in our patients with cystic fibrosis and without vitamin K deficiency. This increase might have been linked to the interference of certain drugs on the enzymatic system of  $\gamma$ -carboxylation of vitamin K dependent factors.

Dr Cornelissen does not say if the eight patients they studied with a normal PIVKA-II concentration received certain drugs (such as antibiotics). However, we agree with his conclusion: vitamin K deficiency is rare in patients with cystic fibrosis supplemented with vitamin K.

- 1 Soulier JP, Gozin D, Lefrère JJ. A new method to assay desgamma-carboxyprothrombin. Results obtained in 75 cases of hepatocellular carcinoma. *Gastroenterology* 1986;91:1258-62.
- 2 Liebman HA, Furie BC, Tong MJ, *et al*. Des-gamma-carboxy (abnormal)-prothrombin as a serum marker of primary hepatocellular carcinoma. *N Engl J Med* 1984;310:1427-31.
- 3 Lefrère JJ, Armengaud D, Leclercq M, Guillaumont M, Gozin D, Alagille D. Des-gamma-carboxyprothrombin and hepatoblastoma. *J Clin Pathol* 1988;41:356.
- 4 Nelsestuen GL, Zytovicz TH, Howard JB. The mode of action of vitamin K: identification of gamma-carboxyglutamic acid as a component of prothrombin. *J Biol Chem* 1974;249:6347-50.
- 5 Bechtold H, Andrassy K, Janchen E. Evidence for impaired hepatic vitamin K<sub>1</sub> metabolism in patients treated with N-methyl-thiotetrazole cephalosporins. *Thromb Haemostas* 1984;51:358-61.
- 6 Lefrère JJ, VanDreden P, Samama P. Elevation of different des-gamma-carboxyproteins in hepatocellular carcinoma. *Thromb Haemostas* 1987;58:1092.

### Modifying the request behaviour of clinicians

In their recent paper, Gama *et al*<sup>1</sup> report that feedback of laboratory data to clinicians "modified" their request behaviour for clinical chemistry and haematology tests (reduced the numbers of tests requested). The authors clearly imply that this modification was a good thing. Their opening sentence refers to "... strategies for improving (my emphasis) laboratory use", and in their discussion they state that their results support the view "that the physicians had become more judicious (my emphasis) in their laboratory use".

In a partly similar study Bareford and Hayling<sup>2</sup> sent each consultant a monthly statement of use of the laboratory by his firm, compared with that of other clinicians. This practice, with three other interventions, they concluded, resulted in a "definite and sustained reduction in inappropriate (my emphasis) requests for laboratory investigations".

In my opinion, both groups are falling into the trap of making unjustified value judgements based on evidence for only one half of the equation: less tests = better/no worse treatment.

Modifications in clinicians' laboratory testing behaviour patterns can only be regarded as desirable or "judicious" if the new modified behaviour can be demonstrated to improve patient care, or at least to result in no worse care. Similarly, requests can only be claimed to be "inappropriate" if it is shown that leaving them out over a period results in no deterioration of clinical care. Unfortunately, it is almost impossible to measure the quality of patient care in the short term. Misdiagnoses, or inappropriate treatment resulting from laboratory tests not being done, could only be detected by worsened morbidity or mortality figures obtained over a period of years. Neither Gama *et al* nor the other group of authors cited above provide any such data to show that their clinicians were not giving their patients worse care than before as a result of curtailing their laboratory requests. In fact, Gama *et al*'s statement that as a result of their initiative "fewer outpatients were investigated, and when investigated had fewer tests performed on them" would suggest, prima facie, that these patients were receiving worse medical treatment than before.

T E BLECHER  
Haematology Department  
University Hospital, Nottingham NG7 2UH

- 1 Gama R, Nightingale PG, Broughton PMG, *et al*. Modifying the request behaviour of clinicians. *J Clin Pathol* 1992;45:248-9.
- 2 Bareford D, Hayling A. Inappropriate use of laboratory services: long term combined approach to modify request patterns. *Br Med J* 1990;301:1305-7.

Dr Gama *et al* comment:

There is ample evidence that many laboratory investigations may be unnecessary for adequate patient care<sup>1-4</sup> and that the recent increased laboratory use has not been associated with an improvement in patient outcome.<sup>5-7</sup> In our study,<sup>8,9</sup> unlike Blecher, we made no unfounded assumption about the quality of patient care. Although we were unable to assess clinical outcome: we agree with Blecher that this, in practice, would be almost impossible to achieve. We believe it unlikely that the reduction in laboratory use through more thoughtful and discretionary ("judicious") testing adversely affected patient management. The fact that fewer outpatients were investigated suggests a reduction in venepunctures (considered unnecessary by the attendant physician) and this, contrary to Blecher's assertion, represents an improvement in the quality of patient care.

Motivation for improving laboratory use should not be limited to better quality of patient care but should also include more efficient use of laboratory and clinical resources.<sup>10,11</sup> We believe that this involves tackling not only laboratory overuse<sup>12</sup> but also underuse<sup>1</sup> and misuse. Gama R, Pickford R, Jones SR, McCauley B, Peters M. Proceedings of the ACB national meeting, 1990:63.

- Hampton JR, Harrison MJG, Mitchell JRA, Prichard JS, Seymour C. Relative contributions of history-taking, physical examination, and laboratory investigation to diagnosis and management of medical outpatients. *Br Med J* 1975;2:486-9.
- Sandler G. Do emergency tests help in the management of acute medical admissions? *Br Med J* 1984;289:973-7.
- Stilwell JA, Young D, Cunningham A. Evaluation of laboratory tests in hospitals. *Ann Clin Biochem* 1980;17:281-6.
- White AJ, Barraclough B. Benefits and problems of routine laboratory investigations in adult psychiatric admissions. *Br J Psychiatry* 1989;155:65-72.
- Carmalt MHB, Whitehead TP. Patient investigation by biochemical profile. *Proc Roy Soc Med* 1971;64:1257-9.
- Griner PF, Liptzin B. Use of the laboratory in a teaching hospital. Implications for patient care, education, and hospital costs. *Ann Intern Med* 1971;75:157-63.
- Martin SP, Donaldson MC, London CD, Peterson OL, Colton T. Inputs into coronary care during 30 years. A cost effectiveness study. *Ann Intern Med* 1974;81:289-93.
- Gama R, Nightingale PG, Broughton PMG, et al. Feedback of laboratory usage and cost data to clinicians: Does it alter requesting behaviour? *Ann Clin Biochem* 1991;28:143-9.
- Gama R, Nightingale PG, Broughton PMG, et al. Modifying the request behaviour of clinicians. *J Clin Pathol* 1992;45:248-9.
- Mutimer D, McCauley B, Nightingale P, Ryan M, Peters M, Neuberger J. Computerised protocols for laboratory investigation and their effect on use of medical time and resources. *J Clin Pathol* 1992;45:572-4.
- Gama R, Broughton PMG, Nightingale P. Audits improve test use. *Clin Chem News* 1990;7:14-6.
- Gama R, Swain DG, Nightingale PG, Buckley BM. The effective use of cardiac enzymes and electrocardiograms in the diagnosis of acute myocardial infarction in the elderly. *Postgrad Med J* 1990;66:375-7.
- Labib M, Gama R, Marks V. Predictive value of D-xylose absorption test and erythrocyte folate in adult coeliac disease: a parallel approach. *Ann Clin Biochem* 1990;27:75-7.

### AgNOR technique in relation to colorectal neoplasia

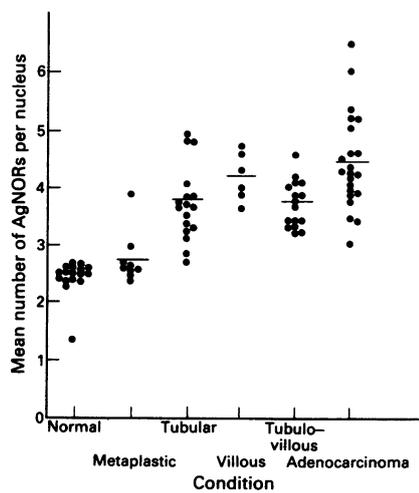
We read with interest the recent paper by Beer *et al*<sup>1</sup> because we have also applied this technique to intestinal tissue. This paper is one of a selection that has been published on this topic in recent years in which authors express varied enthusiasm for the method.<sup>2-5</sup>

We examined 91 surgical resection specimens of large bowel comprising normal mucosa (n = 10); tubular (n = 18), villous (n = 6) and tubulo-villous (n = 16) adenomas; and moderately differentiated adenocarcinomas (n = 22). The batch of malignant tumours comprised five Dukes' A, six B, and 11 C. The method used was as described by Smith and Crocker,<sup>6</sup> except that the staining time was one hour; 100 cells were counted.

The results are shown in the table. An unpaired *t* test was applied to the data and a highly significant difference (*p* = 0.001) was found between normal mucosa and both the adenomatous polyps and the adenocarcinomas. No statistical difference existed between normal and metaplastic, or between benign

#### Summary of results

Condition	Mean (SD) AgNOR count
Normal mucosa	2.37 (0.28)
Metaplastic polyp	2.71 (0.44)
Tubular adenoma	3.67 (0.64)
Villous adenoma	4.12 (0.43)
Tubulo-villous adenoma	3.62 (0.41)
Adenocarcinoma	4.34 (0.86)



and malignant conditions. AgNOR numbers varied enormously in all but the normal and metaplastic states, this being most pronounced in malignancy (figure). No correlation was observed between the AgNOR number and the Dukes' stage, although we acknowledge that relatively few of each stage were examined.

In terms of diagnostic usefulness, our results for colorectal tissue agree with those of Beer *et al*<sup>1</sup> and Yu *et al*<sup>5</sup> for stromal tumours of the stomach and small intestine. This contrasts with the findings of Yang *et al*,<sup>3</sup> who discriminated colonic tubular and villous adenomas from adenocarcinomas. Surprisingly, Griffiths *et al*<sup>2</sup> could find no link between AgNOR number and neoplasia in large bowel tissue. Unlike us, Ofner *et al*<sup>4</sup> established a correlation between AgNOR number and Dukes' staging.

Clearly, AgNOR number is a reflection of increased cell proliferation and may be used to distinguish normal tissue from neoplastic. However, as an accurate discriminator of malignancy, this technique is inadequate when applied to intestinal tissues. We feel that it offers little more than the haematoxylin and eosin preparation in the area of large bowel diagnostic pathology.

AJ WOOD  
MJ CONNOCK  
School of Applied Sciences,  
Wolverhampton Polytechnic,  
Wolverhampton WV1 1SB  
CA ALLEN  
RH GRACE  
Departments of Pathology and Surgery,  
The Royal Hospital, Wolverhampton

- Beer TW, Rowlands DC, Crocker J. AgNOR counts and determination of malignancy in stromal tumours of the stomach and small intestine. *J Clin Pathol* 1992;45:172-4.
- Griffiths AP, Butler CW, Roberts P, Dixon MF, Quirke P. Silver stained structures (AgNORs), their dependence on tissue fixation and absence of prognostic relevance in rectal adenocarcinoma. *J Pathol* 1989;159:121-7.
- Yang P, Huang GS, Zhu XS. Role of nucleolar organiser regions in differentiating malignant from benign tumours of the colon. *J Clin Pathol* 1990;43:235-8.
- Ofner D, Totsch M, Sandbichler P, et al. Silver stained nucleolar organiser region proteins (AgNORs) as a predictor of prognosis in colonic cancer. *J Pathol* 1990;162:43-9.
- Yu CCW, Fletcher CDM, Newman PL, Goodlad JR, Burton JC, Levison DA. A comparison of proliferating cell nuclear antigen (PCNA), immunostaining, nucleolar organiser region (AgNOR) staining and histological grading in gastrointestinal stromal tumours. *J Pathol* 1992;166:147-52.
- Smith R, Crocker J. Evaluation of nucleolar organiser region-associated proteins in breast malignancy. *Histopathology* 1988;12:113-25.

### Breast biopsy specimen fixation

Further to the correspondence by Drs Start, Cross, and Smith<sup>1</sup> regarding the procedure of fixing breast biopsy specimens, we add our findings to this debate.

In our view the handling of this kind of specimen poses a dilemma: for best slicing and minimisation of distortion for assessment of resection margins and extent of lesion, the specimen should be fixed before slicing. To overcome this problem we suggest that the specimen should be injected with 10% neutral buffered formalin on receipt then left to fix for 24 hours before slicing.

We use a 10 ml syringe with a 21 gauge needle. The amount of formalin injected depends on the size of the specimen. The injection can be performed by technical staff, which means the specimen need not be sent dry and the pathologist does not have to be on hand when the specimen is received: this may often be the case in a district general hospital.

This technique offers adequate fixation of tissues deep within the specimen while allowing fixation of the outside which "hardens" the specimen, giving optimal slicing.

There are two possible hazards that need to be borne in mind when using this technique. The first is the danger of needlestick injury; the second concerns the splashback of formalin which can occur if too much pressure is applied, particularly when injecting firm areas of tissue. Accordingly, appropriate protective clothing should be worn and great care taken when performing this procedure.

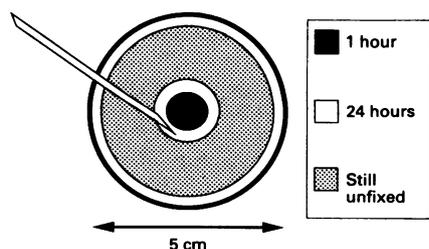
We have found a definite improvement using this method in the quality of morphology in subsequent sections compared with those from specimens which were allowed to fix overnight before slicing and were not injected.

We propose that this method helps reduce the inevitable variation in fixation that occurs with these specimens, and thereby reduces the associated variation in mitotic counts which may affect grading.<sup>2</sup> It also improves assessment of resection margins and extent of lesions.

We accept that our findings are subjective and anecdotal, but feel that there is sufficient benefit to merit extending the use of this procedure from localisation biopsy specimens and wide local excision specimens to mastectomy specimens.

EP RUBAN  
W SUMNALL  
M STEPHENS  
Department of Histopathology,  
Central Pathology Laboratory,  
Hartshill, Stoke on Trent ST4 7PA

- Start RD, Cross SS, Smith JMF, et al. Standardisation of breast tissue fixation procedures. *J Clin Pathol* 1992;45:182.
- Start RD, Cross SS, Smith JMF *et al*. *J Pathol* 1991;163:154a.



Penetration of 10% neutral buffered formalin into an unsliced 5 cm diameter lump after a single 1 ml injection of fixative.