

Table 2 Categories between which significant differences in AgNOR counts were observed ( $p \leq 0.05$ )

Between	Normal	and	CIN III
Between	Normal	and	invasive carcinoma
Between	Atrophic	and	CIN II
Between	Atrophic	and	CIN III
Between	Atrophic	and	invasive carcinoma
Between	Chronic cervicitis	and	CIN III
Between	Chronic cervicitis	and	invasive carcinoma
Between	Metaplastic	and	CIN III
Between	Metaplastic	and	invasive carcinoma
Between	HPV	and	CIN III
Between	HPV	and	invasive carcinoma
Between	CIN I	and	invasive carcinoma

The difference between our findings and those of the previously published study suggests that there is a need for standardisation of counting procedures. It also suggests that there could be a high interobserver error. This is supported by the fact that we found a high inter- and intra-observer error in our study—9.4% and 8.8%, respectively.

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Reference

- Rowlands DC. Nucleolar organising regions in cervical intraepithelial neoplasia. *J Clin Pathol* 1988;41:1200-2.

Dr Rowlands comments:

My study was limited to investigating the possible value of the AgNOR technique in the diagnosis of the different grades of cervical intraepithelial neoplasia (CIN). Ramsden *et al* have independently drawn the same conclusion that I did in my published study—that the NOR changes are small and do not add to the information gained by examining the morphological changes seen on sections stained routinely with haematoxylin and eosin.

Ramsden *et al*, however, seem very concerned that their numerical results of AgNOR counting differ from mine. Unfortunately, they do not give their numerical results, so it is difficult to make any specific comment, but some general remarks need to be made. Many different factors may affect the AgNOR count. It has been shown that different fixatives affect the results of the AgNOR stain<sup>1</sup> and variations in the processing of tissue may also have an effect. Variations in the thickness of sections will obviously affect the AgNOR count, higher counts being expected with increasing thickness of section. The length of time that

sections are stained affects the visibility of AgNORs—longer staining shows up more small AgNORs but makes it more difficult to determine the number of AgNORs making up a larger clump.<sup>2</sup> It is also possible that differences in ambient temperature and in the reagents, particularly gelatin, used by different laboratories are important. Thus care must be taken when comparing absolute counts of similar lesions performed at different centres.

In fact, I suspect that none of these factors is important in explaining the differences between the two studies. Ramsden *et al* counted AgNORs at levels of the ectocervical epithelium which differed from those I studied. As AgNOR counts were observed to vary at certain levels of the epithelium, this difference in counting method probably explains the difference in counts that Ramsden *et al* observed.

Dr Ramsden and her colleagues call for the standardisation of the AgNOR counting procedures. This problem has been recently addressed by Crocker, Egan, and Boldy.<sup>3</sup> Difficulties in AgNOR counting, however, tend to occur in lesions where the AgNOR count is high. This was not the case in these two studies in which low AgNOR counts were found. More difficult still is the problem of standardisation of the staining technique and handling of specimens to be studied—this is currently being investigated.

The other problem raised by Dr Ramsden refers to the changes in AgNOR count as the grade of CIN increases. My study was intended to investigate the possible usefulness of the technique in diagnosis. I found a significant difference between CIN III and the lower grades of CIN and normal epithelium. Ramsden *et al* also showed a significant difference between CIN III and normal epithelium, but found intermediate counts for CIN I and II. This is supported by the study recently published by Egan *et al*.<sup>4</sup> If AgNOR counts are taken to reflect differences in proliferation then the change in AgNOR count with different grades of CIN

is of interest, but as the present studies have only shown a small difference in AgNOR counts, a much larger study will be needed to assess the true difference among the various grades of CIN.

References

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Use of nucleolar organiser regions (NOR's) for diagnosing gynaecological neoplasia

I read with interest the article by Dr Rowlands<sup>1</sup> as I have recently applied the technique to a wide variety of gynaecological conditions. The material and methods used were identical to those used by Rowlands. Some of my findings are summarised in table 1.

I studied some 25 cases comprising eight normal junctional cervixes, three CIN I, eight CIN II, and eight CIN III. Although both investigations show a similar trend in terms of AgNOR number, the results differed considerably in magnitude, most importantly in the cases of normal tissue and CIN III (table 2). This perhaps serves to emphasise the degree of ambiguity which can arise during the counting procedure, particularly with respect to the large number of AgNOR aggregates present in CIN III. Having established normality, the "analysis of variance technique" indicated a significant difference between normal tissue and cases of CIN and between CIN III and the other two CIN grades. The AgNOR technique as used here, however, cannot be used to distinguish between CIN I and II. The important finding is that on the basis of AgNOR counts I was able to distinguish cases of CIN from normal junctional cervix and to show very effectively cases of CIN III.

An important difference between the method used by Rowlands and my own was that the former counted AgNOR's in the basal half of the squamous epithelium while I