

DISCRIMINATION OF HEPATOCELLULAR CARCINOMA FROM CIRRHOTIC NODULES BY AgNOR STAIN

ALI ZARE MEHRJARDI, M.D.,* AND
MEHDI MASSOUMIAN, M.D.**

From the Department of Pathology, Kerman University of Medical Sciences, Kerman, I.R. Iran.

ABSTRACT

Discrimination of regenerative cirrhotic nodules of the liver (CN) from hepatocellular carcinoma (HCC) is sometimes difficult. We examined the utility of AgNOR staining in this context. Fifteen cases of HCC and 25 cases of CN were stained by AgNOR method and the mean AgNOR number for one hundred nuclei in each case was determined. There was a significant difference between the mean AgNOR counts in CN (6.63 ± 4.00) and HCC (11.36 ± 2.88) ($p < 0.0005$).

Discriminant analysis showed that all HCC cases and over 90% of CN could be correctly distinguished by this method. It seems that AgNOR staining is an accurate and readily available tool for differentiating CN from HCC.

MJIRI, Vol. 12, No. 4, 329-331, 1999

Keywords: Cirrhosis, Cirrhotic nodule, Hepatocellular carcinoma, AgNOR.

INTRODUCTION

Primary cancer of the liver (HCC) is a common malignancy, currently being the most common malignant tumor (excluding skin), and the most common cause of death from cancer worldwide.¹ Cirrhosis has long been considered a strong predisposing factor for the development of HCC, and the risk increases with the duration of the disease.^{1,2} In this regard, distinction of a regenerative cirrhotic nodule (CN) from HCC is often difficult for the surgical pathologist, especially in small needle biopsy specimens. A number of modalities have been employed for this purpose, e.g., flow cytometry, digital image analysis, and AgNOR counting.^{2,3}

As AgNOR staining can be readily performed in almost every histopathology laboratory, we tried to examine the usefulness of this method for discrimination of HCC and CN.

*Assistant Professor of Pathology.

**Resident in training.

MATERIALS AND METHODS

The files of the Department of Pathology of Hospital No. 1, Kerman, were searched from 1989 to 1995, and 334 liver samples were found. Of these, 15 cases of HCC and 25 cases of hepatic cirrhosis were eventually selected for study after reviewing the available slides. Only cases with adequate tissue blocks were chosen. These were cut to 4 microns thickness and stained by AgNOR method as described by Bancroft.⁴ AgNORs were clearly visible as small or large black dots within the nucleus and the nucleolus (Figs. 1,2). In each case, AgNOR dots were counted under oil immersion ($\times 1000$) in one hundred nuclei in the most active areas of the lesions. All discernible dots, whether intranucleolar or not, were individually counted, as recommended by Crocker et al.,⁵ and the mean number of AgNOR was calculated for each case.

Statistical analysis included Student's two-tailed t-test for analysis of variance; discriminant analysis using Fisher's linear discriminant function; and Inter-rater agreement test to evaluate the discriminatory power of mean AgNOR numbers.

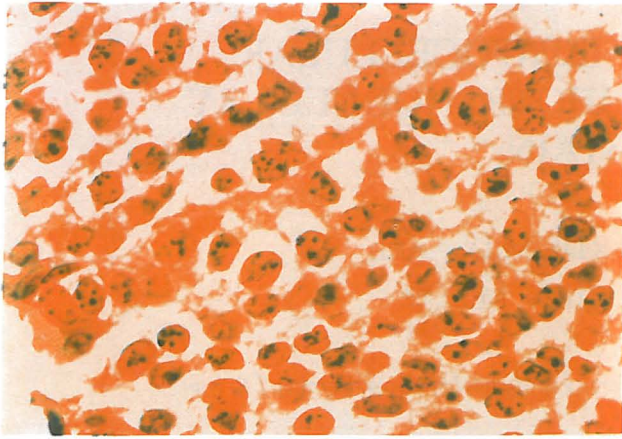


Fig. 1. Cirrhotic nodule, AgNOR stain. NORs are clearly visible as black dots (×400).

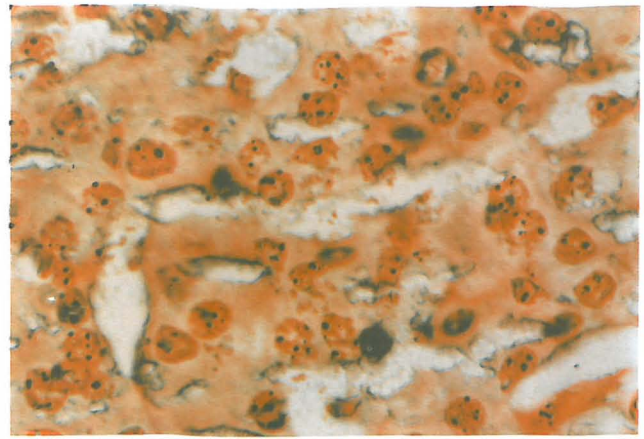


Fig. 2. Hepatocellular carcinoma, AgNOR stain (×400).

Table I. Average number of AgNOR in cirrhotic nodules and hepatocellular carcinoma.

| Histology | Number of Cases | Mean(±SD) | Range |
|--------------------------|-----------------|------------|------------|
| Cirrhotic nodule | 25 | 6.63±4.00 | 3.36-15.43 |
| Hepatocellular carcinoma | 15 | 11.36±2.88 | 8.95-19.93 |

Table II. Discriminant analysis of AgNOR counts in cirrhotic nodules (CN) and hepatocellular carcinoma (HCC).

| Histology | CN | HCC | Correct Discrimination(%) |
|--------------------------|----|-----|---------------------------|
| Cirrhotic nodule | 23 | 2 | 92 |
| Hepatocellular carcinoma | 0 | 15 | 100 |

RESULTS

A total of 15 HCC and 25 cirrhosis cases were studied. The median age of the patients with HCC was higher (54.5 years, range 20-90 years) than that of patients with cirrhosis (33 years, range 2 months-74 years). The male to female ratio was 6.5: 1 for HCC cases and 3:2 for cirrhotic patients.

Results of AgNOR counting in these groups can be found in Table I. The scattergram of the mean number of AgNOR per nucleus in the cases studied is shown in Figure 3. Statistical analysis showed that there is a significant difference in the mean number of AgNORs between cirrhotic nodules and HCC ($p < 0.0005$). Discriminant analysis of the results were also encouraging (Table II); over 90% of cirrhotic nodules and all HCCs were correctly classified. By inter-rater agreement test and evaluation of the kappa

value, the percentage of diagnostic agreement was calculated as 0.9 (90%), corresponding to the "very good" category.

DISCUSSION

In hepatic cirrhosis, regenerative nodules show increased cellularity of liver cell plates and mild to moderate degrees of cellular pleomorphism.⁶ Similar findings are commonly seen in better differentiated forms of HCC.⁷ The clinical setting of these diseases is not helpful for their separation. Castaldo et al.⁸ have identified serum biochemical parameters that can discriminate cirrhosis from HCC. It is debatable whether these tests are useful when a developing HCC is in early stages of growth and still amenable to therapeutic interventions, or only when it has already grown to a large size.

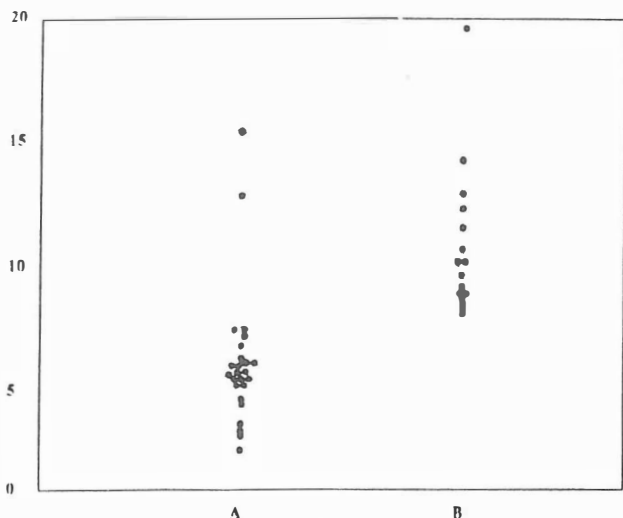


Fig. 3. Scattergram of actual AgNOR counts in the study groups. A: Cirrhotic nodules, B: Hepatocellular carcinoma.

Morphometric techniques including flow cytometric DNA analysis and immunostaining for proliferating cell nuclear antigen (PCNA) have been useful for differentiation of cirrhotic nodules from HCC.^{3,9} However, these modalities are not routinely available in all histopathology laboratories. AgNOR staining has been very promising for differentiation of benign and malignant lesions of a large number of organs, including the liver.^{9,12} It is well established that the number of AgNORs is greater in malignant tumors than in their benign counterparts or in normal tissues.^{9,13} Crocker and McGovern showed that HCC was characterized by a greater quantity of AgNOR than cirrhotic nodules.¹¹ Our study further confirms this statement. Furthermore, analysis of variance of our results shows that the difference between the means in our study groups is quite significant.

A point to be addressed in assessing AgNOR numbers is the counting method. Obviously this has a significant effect on results. Crocker et al.⁵ discuss this problem and conclude that counting all discernible dots—whether in nucleolar or not—give superior results for discrimination of benign and malignant lesions. We adopted this method, though admittedly it is more tedious and time consuming. This explains the large difference between the results of our study and that of others. For example, mean \pm SD AgNOR counts for early HCC and CN in the study of Aoki et al.¹³ were 1.72 ± 0.18 and 1.35 ± 0.08 , respectively. Similar values in the study of Shimizu et al.¹⁴ were 3.26 ± 1.23 and 1.49 ± 0.14 . The much greater difference between the mean AgNOR counts of our study groups shows higher accuracy and less chance of overlap between the groups when our counting method is used.

In conclusion, our results further confirm the utility of AgNOR counting for discrimination of HCC and cirrhotic

nodules. It is interesting to learn about the AgNOR status of other benign and borderline tumors of the liver, and the range of AgNOR counts for HCC and CN in future studies.

REFERENCES

1. Bloustein PA: Surgical diseases of the liver. In: Silverberg SG (ed.), Principles and Practice of Surgical Pathology. 2nd ed., New York: Churchill-Livingstone Co., pp. 1317-1346, 1990.
2. Barwick KW, Rosal J: Liver. In: Rosai J, (ed.), Ackerman's Surgical Pathology. 8th ed., St. Louis: Mosby Co., pp. 857-942, 1996.
3. Rua S, Comino A, Fruttero A, et al: Flow cytometric DNA analysis of cirrhotic liver cells in patients with hepatocellular carcinoma can provide a new prognostic factor. *Cancer* 78(6): 1195-1202, 1996.
4. Bancroft JD, Stevens A: Theory and Practice of Histological Techniques. 3rd ed., New York: Churchill-Livingstone Co., p. 643, 1990.
5. Crocker J, Boldy DA, Egan MJ: How should we count AgNOR? Proposal for a standardized approach. *J Pathol* 158: 185-188, 1989.
6. Wright D, Paradinas F: Liver, biliary tract and exocrine pancreas. In: Symmers WStC (series ed.), Systemic Pathology. 3rd ed., Vol. 11, New York: Churchill-Livingstone Co., pp. 469-542, 1994.
7. Ferrell L: Malignant liver tumors that mimic benign lesions: analysis of five distinct lesions. *Semin Diag Pathol* 12: 64-76, 1995.
8. Castaldo G, Oriani G, Lofrano MM, et al: Differential diagnosis between hepatocellular carcinoma and cirrhosis through a discriminant function based on results of serum analyses. *Clin Chem* 42(8 Pt 1): 1263-9, 1996.
9. Editorial. NORs-A new method for the pathologist. *Lancet* June 20, 1413-4, 1987.
10. Ruschoff J, Prasser C: Diagnostic value of AgNOR staining in follicular cell neoplasms of the thyroid. *Am J Surg Pathol* 17: 12, 1281-8, 1993.
11. Crocker J, McGovern J: Nucleolar organizer regions in normal, cirrhotic and carcinomatous livers. *J Clin Pathol* 41: 1044-8, 1988.
12. Crocker J: Nucleolar organizer regions (NOR) in neoplasms. In: McGee J, Isaacson PG, Wright NA, (eds.), Oxford Textbook of Pathology. New York: Oxford University Press, pp. 586-90, 1992.
13. Aoki K, Sakamoto M, Hirohashi S: Nucleolar organizer regions in small nodular lesions representing early stages of human hepatocarcinogenesis. *Cancer* 73(2): 289-93, 1994.
14. Shimizu K, Izumi R, Ii T: Prognostic significance of nucleolar organizer regions in hepatocellular carcinoma. *Hepatology* 21(2): 393-7, 1995.

