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Argyrophilic nucleolar organizer region counts in multiple myeloma: a histopathological study on bone marrow trephine biopsies

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Summary. Argyrophilic nucleolar organizer region (AgNOR) analysis was performed on bone marrow biopsies from 90 patients with multiple myeloma (MM) at presentation. The pattern of AgNOR expression and its relationship to histological features were evaluated. The mean AgNOR number per plasma cell was directly correlated with the degree of MM differentiation (3.18 for G1, 4.36 for G2, 6.13 for G3; $P<0.0001$), with the percentage of bone marrow plasma cells (BMPC%) (3.06 for BMPC% ≤ 20, 4.28 for BMPC% 21–50, 5.14 for BMPC% >50; $P<0.0001$), with the pattern of medullary involvement (3.63 for interstitial, 4.44 for nodular, 5.17 for diffuse involvement; $P<0.001$) and with medullary fibrosis (5.23 for cases with fibrosis, 4.29 for cases without fibrosis; $P<0.05$). The plasma cells of G1 MM showed 2–3 large AgNORs, tightly grouped in a central nuclear cluster; those of G2 MM showed a central nuclear cluster composed of 4–5 medium-size dots and/or two clusters of 2–3 dots; the G3 MM plasma cells showed many small dots scattered in the nucleolus or dispersed in the nucleus. Our results indicate the diagnostic value of AgNOR analysis in MM and suggest the use of this method for identifying clones of atypical plasma cells with different proliferative activity in bone marrow biopsies. It allows simultaneous evaluation of the morphology and kinetics of MM cells in routinely fixed, decalcified, paraffin-embedded material.

Key words: Argyrophilic nucleolar organizer region – Multiple myeloma – Diagnosis – Bone marrow biopsies

Introduction

Multiple myeloma (MM) is one of the most frequent neoplastic disorders of the bone marrow. The morphological diagnosis of MM is still based on an arbitrarily selected percentage of plasma cells (PCs) in bone marrow aspirates (Oken 1984), and a histological classification has not yet been widely accepted. However, the relevance of the morphology of MM cells in diagnosis and prognosis has been repeatedly demonstrated in large series of bone marrow biopsies (Bartl et al. 1982, 1984a, b, 1985, 1987; Bartl and Frisch 1989).

Recently, analysis of the nucleolar organizer regions (NORs) has been introduced into surgical pathology, as a simple one-step argyrophilic technique (AgNOR method) can be applied to routinely fixed and paraffin-embedded tissue sections (Ploton et al. 1986). Different NOR patterns provide information about nucleolar structure and activity in hyperplastic and neoplastic conditions (Walker 1988) and may be useful for distinguishing benign and malignant cells. Small size, large number and scattered distribution of NORs are characteristic of malignant tumours; large size, small number and clustered distribution are characteristic of benign tumours (Crocker and Nar 1987; Crocker and Skilbeck 1987; Derenzini et al. 1988b).

It has also been shown that the AgNOR method can provide information on the proliferative activity of neoplastic cells. In non-Hodgkin's lymphomas (Hall et al. 1988) and breast carcinomas (Dervan et al. 1989) there is a clear correlation between AgNOR staining and Ki-67 immunostaining. In non-Hodgkin's lymphoma (Crocker et al. 1988) and breast tumours (Giri et al. 1989) there is a relationship between NOR number and percentage of S-phase cells determined by DNA flow cytometry. In meningiomas there is a correlation between NORs and bromodeoxyuridine (BrdU) incorporation (Orita et al. 1990); a linear relationship was found between cell duplicational activity, evaluated by tritiated thymidine incorporation, and the amount of AgNOR proteins in cell lines derived from different tumour types (Derenzini et al. 1990b).

We have investigated bone marrow trephine biopsies from 90 patients with MM at presentation, using AgNOR staining. We have compared the histological char-

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acteristics of the bone marrow with AgNOR counts and patterns to see if the technique was of diagnostic value in MM, and whether AgNOR patterns correlate with other morphological features.

Materials and methods

We studied bone marrow trephine biopsies from 90 patients with MM at presentation. The diagnosis was established according to the criteria of the Chronic Leukaemia-Myeloma Task Force (1973). All the cases were confirmed clinically and immunologically; laboratory procedures included bone marrow aspirate, serum and urine electrophoresis and immunoelectrophoresis, determination of complete blood counts, serum albumin, creatinine, and calcium. Forty patients were female, 50 male; the mean age was 63.4 years (42–87 years).

Yamshidi needle biopsies from posterior-superior iliac crest were immediately fixed in buffered acid formol or Bouin's for 24 h and immersed in 70% ethanol for at least 15 min. Bouin's solution, which is not the optimal fixative for AgNOR staining (Derenzini et al. 1988a), was the only available fixative for 15 of our cases. The specimens were then decalcified in Mielodec (Bioptica) (EDTA, HCl mixture) for 6 h, routinely dehydrated and embedded in paraffin.

Sections 3 μm thick were stained with haematoxylin and eosin, periodic acid-Schiff (PAS) and Perl, Dominici and Gomori stains. Only sections measuring at least 8×2 mm, with a minimum marrow area (excluding periosteal tissues, cortical bone and artefacts) of 9 mm^2 were accepted. All biopsies were independently assessed by two of the authors (R.N. and A.P.) and the following variables were evaluated:

1. Percentage of bone marrow plasma cells (BMPC%) determined as the differential of at least 2000 nucleated bone marrow cells at $\times 480$ magnification.
2. Type of BMPC infiltration: interstitial (PCs loosely dispersed with preservation of bone marrow structure); nodular (aggregates of more than 10 PCs dispersed in individual marrow spaces); diffuse (PCs replacing the haemopoietic tissue and fat cells, with generalized or patchy changes in bone marrow structure).
3. BMPC atypia: G1 (PCs normal or with slight atypia and non-prominent nucleolus); G2 (PCs with moderate atypia, small prominent nucleoli, polymorphism, binuclearity); G3 (PCs with severe atypia, large and very prominent nucleoli, mitoses, and some giant and bizarre PCs).
4. Marrow cellularity (percentage of myeloid tissue in relation to the whole marrow, including adipose, fibrotic or neoplastic tissue, evaluated by morphological criteria). Two classes were considered: $\leq 30\%$ and $>30\%$ (30% being the median of the marrow cellularity of the whole series).
5. Presence of marrow fibrosis (fine or coarse reticulin fibres revealed by Gomori staining).
6. Presence of an "excess" of haemosiderin (more than 3 Perl's-positive particles per field at $\times 400$ magnification).
7. Presence of "reactive" lymphoid nodules, greater than 50 μm in diameter, composed of small lymphocytes.

For AgNOR staining sections 3 μm thick were cut, dewaxed in xylene, transferred to absolute ethanol, post-fixed with acetic acid-ethanol (1:3) for 5 min, rinsed with absolute ethanol, passed through alcohols and then rehydrated. The staining was done using a solution consisting of one volume of 2% gelatin in 1% aqueous formic acid and two volumes of 50% silver nitrate, at 37° C for 10–12 min. Slides were then treated for 1 min in 5% aqueous sodium thiosulphate, rinsed in distilled water, counterstained with methyl green and mounted in resin. Controls included sections of renal cell carcinoma known to have high AgNOR counts (Pich et al. 1991). In addition, an effective internal control was provided by the scattered lymphocytes that on average had one single silver-stained dot.

The specimens were examined using a $\times 100$ oil immersion lens. Black dots within nuclei from at least 100 myeloma cells were counted in each case. Single AgNORs and individual AgNORs within clumps were counted by careful focusing through the whole thickness of the section. When large, polycyclic structures (overlapping NORs) were observed, they were counted as a single AgNOR if individual AgNORs could not be identified. The mean number of AgNORs per nucleus was calculated.

Correlation between AgNOR counts and age, sex, percentage of BMPC, type of BMPC infiltration, PC cytology, marrow cellularity, fibrosis, excess of haemosiderin and presence of lymphoid nodules was estimated by one-way analysis of variance.

Results

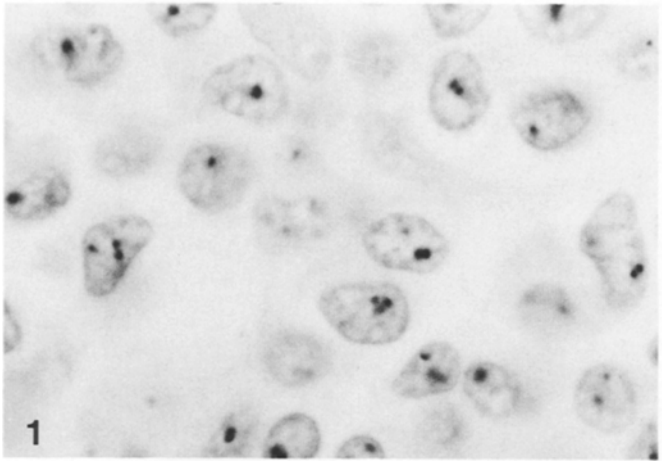
The mean AgNOR number per PC for the whole series was 4.44 (2.15–9.95). No difference was seen according to sex (4.30 for females, 4.54 for males; $P=0.45$) or age (5.24 for patients ≤ 45 years, 4.34 for patients 46–70 years, 4.44 for patients older than 70 years; $P=0.29$).

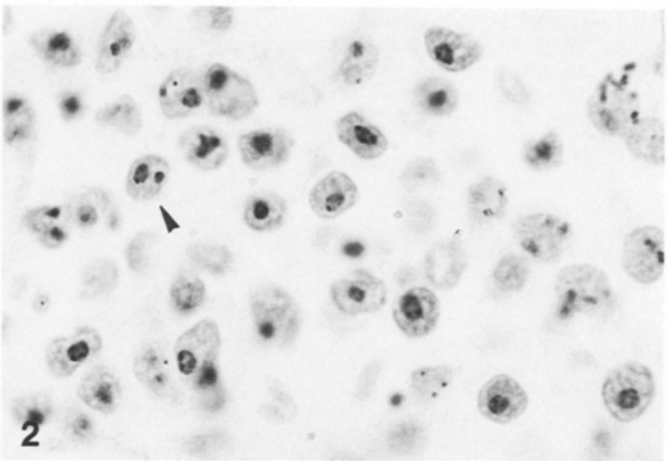
A highly significant association was found between AgNOR number and percentage of BMPC (3.06 for BMPC $\leq 20\%$, 4.28 for BMPC 21–50%, 5.14 for BMPC $>50\%$, $P<0.0001$). The mean AgNOR number per PC for the interstitial pattern of invasion was 3.63, for the nodular 4.44, for the diffuse 5.17, ($P<0.001$). For the G1 MM it was 3.18, for the G2 4.36, for the G3 6.13, ($P<0.0001$). In the well-differentiated G1 MM, the PC nucleus showed AgNORs tightly grouped in a central cluster composed of 2–3 relatively large dots (Fig. 1); in the G2 MM a central cluster of 4–5 medium-sized dots was usually observed, and two clusters composed of 2–3 medium-sized dots could also be seen (Fig. 2). The poorly differentiated G3 MM showed many small single dots scattered in the nucleolus or dispersed in the nucleus (Fig. 3). Schematic AgNOR configurations related to PC differentiation are shown in Fig. 4.

Significantly different AgNOR numbers were found in MM with fibrosis (5.23) versus cases without fibrosis (4.29) ($P=0.02$). The mean AgNOR per PC for cases with $\leq 30\%$ marrow cellularity was 4.64 versus 4.11 for cases with $>30\%$ cellularity ($P=0.09$); 4.42 for cases with excess of haemosiderin versus 4.44 for cases without such excess ($P=0.97$); 4.14 for MM with lymphoid nodules versus 4.45 for MM without these ($P=0.61$). The results are summarized in Table 1.

Discussion

AgNOR analysis on bone marrow biopsies in MM showed significant relationships between AgNOR number and PC differentiation: 3.18 for G1, 4.36 for G2 and 6.13 for G3 MM. This is in line with results observed in other human tumours, in which the amount of AgNORs is directly related to the histological degree of malignancy. This is true for non-Hodgkin's lymphomas (Crocker and Nar 1987; Kim et al. 1988), intraepithelial neoplasms of the cervix (Egan et al. 1988a, 1990), neuroblastomas (Egan et al. 1988b), brain tumours (Hara et al. 1990; Plate et al. 1990), breast tu-





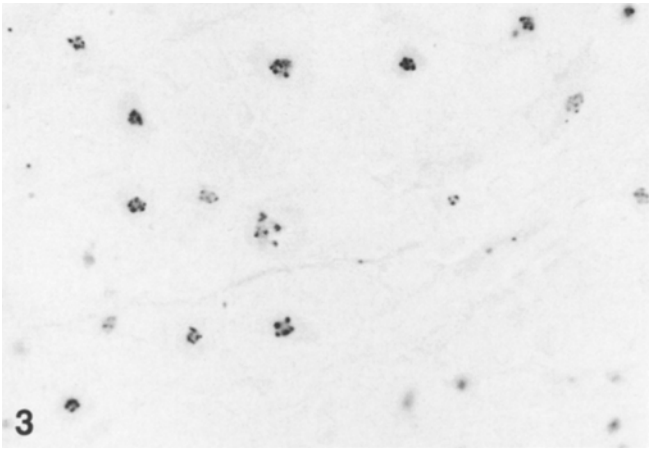


Fig. 1. G1 multiple myeloma (MM): the plasma cells show 2–3 large argyrophilic nucleolar organizer regions (AgNORs) tightly aggregated in central nuclear cluster. AgNOR staining, ×1100

Fig. 2. G2 MM: the plasma cells show 4–5 medium size AgNORs arranged in a central nuclear cluster or two clusters composed of 2–3 dots (*arrow*). AgNOR staining, ×880

Fig. 3. G3 MM: the atypical plasma cells show large numbers and scattered distribution of AgNORs. AgNOR staining, ×750

Fig. 4. Schematic configurations of AgNORs in plasma cells according to the degree of MM differentiation. *A*, G1MM; *B-B'*, G2MM; *C-C'*, G3MM

mours (Derenzini et al. 1990a; Rüschoff et al. 1990), bladder tumours (Cairns et al. 1989; Ooms et al. 1989; Rüschoff et al. 1991) and prostatic tumours (Deschênes and Weidner 1990).

The degree of PC differentiation also correlated with the pattern of AgNOR distribution. In G1 MM AgNORs were mostly arranged in central clusters of 2–3 relatively large dots, while in G3 MM they were small, scattered in the nucleolus or dispersed throughout the nucleus; an intermediate pattern of distribution was observed in G2 MM, where AgNORs could also be arranged in two clusters composed of medium-size dots.

Recently, it has been shown that scattered lymphocytes and PCs in normal bone marrow have only one or two relatively large AgNORs (Nikicicz and Norback 1990). In our cases, the scattered normal lymphocytes, when present, had only one single AgNOR. In the most differentiated MM, the mean AgNOR number per PC was 3.18 (range 2.15–4.45), indicating that the myeloma cells, even if well differentiated, have AgNOR numbers constantly higher than those in normal, morphologically similar PCs. This effect is probably related to the abnormal DNA content of the MM cells, in respect to normal or reactive PCs (Latreille et al. 1980; Barlogie et al. 1983) and/or to the sequential evolution of variant new clones that may appear in all phases of the disease (Durie 1984). These have also been shown to contain excess DNA (Barlogie et al. 1982). AgNOR staining is thus a reliable method of distinguishing normal from neoplastic PCs, especially when their number is minimal and they are well differentiated.

We also found significant correlations between AgNOR counts and percentage of BMPCs (3.06 for BMPC% ≤ 20, 4.28 for BMPC% 21–50, 5.14 for BMPC% > 50), pattern of marrow involvement and medullary fibrosis. These correlations may depend on the relationship between histological variables in MM: in fact plasmablastic MM is generally associated with high BMPC% and a diffuse pattern of infiltration and heavy fibrosis, while plasmocytic MM is associated with

Table 1. Number of argyrophilic nucleolar organizer regions (AgNOR) per plasma cell in bone marrow biopsies of multiple myeloma (MM)

Variables	*n*	Mean	Median	SD	Min.–Max.	*P*
All series	90	4.44	4.28	1.44	2.15–9.95	
Sex M	50	4.54	4.53	1.55	2.15–9.95	
F	40	4.30	3.91	1.28	2.68–8.29	0.45
Age ≤45	7	5.24	4.87	1.04	3.95–6.87	
years 46–70	56	4.34	4.11	1.41	2.15–8.34	0.1
>70	27	4.44	4.58	1.56	2.28–9.95	0.7
Percentage of bone marrow plasma cells						
≤20	17	3.06	2.99	0.7	2.15–4.48	
21–50	33	4.28	4.31	0.8	2.90–6.26	<0.0001
>50	40	5.14	4.77	1.63	2.85–9.95	<0.01
Infiltration						
Interstitial	28	3.63	3.69	1.01	2.15–6.26	
Nodular	31	4.44	4.31	1.37	2.90–9.95	<0.05
Diffuse	31	5.17	4.87	1.47	2.85–8.34	<0.05
Atypia G1	20	3.18	3.05	0.7	2.15–4.45	
G2	53	4.36	4.47	0.92	2.68–6.97	<0.0001
G3	17	6.13	5.73	1.77	3.96–9.95	<0.0001
Cellularity						
≤30%	55	4.64	4.53	1.65	2.20–9.95	
>30%	35	4.11	3.97	0.92	2.15–6.97	0.09
Excess of haemosiderin						
Present	13	4.42	4.45	1.26	2.20–6.97	
Absent	77	4.44	4.23	1.47	2.15–9.95	0.97
Fibrosis						
Present	14	5.23	4.68	1.64	3.43–8.34	
Absent	76	4.29	4.16	1.36	2.15–9.95	0.02
Lymphoid nodules						
Present	6	4.14	3.95	1.27	2.90–6.26	
Absent	84	4.45	4.33	1.45	2.15–9.95	0.61

low BMPC%, interstitial infiltration and slight fibrosis (Bartl et al. 1982, 1987; Riccardi et al. 1990).

All these histological bone marrow characteristics have a significant prognostic value in MM (Bartl et al. 1982, 1987; Carter et al. 1987; Paule et al. 1987, 1989). As we have shown that these patterns are correlated with AgNOR counts, AgNOR counts should also be of prognostic value in MM.

It has also been shown that the AgNOR method can provide information on the proliferative activity of neoplastic cells in many human tumours (Crocker et al. 1988; Hall et al. 1988; Dervan et al. 1989; Giri et al. 1989; Derenzini et al. 1990b; Orita et al. 1990). In MM the proliferative activity of neoplastic cells has been evaluated with tritiated thymidine or BrdU incorporation, in PCs obtained from bone marrow aspirates that may not reflect the actual proportion or characteristics of the entire neoplastic population, since fibrosis, non-homogeneous PC distribution or dilution can affect the aspirates (De Gramont et al. 1984; Krzyzaniak et al. 1988).

Finally, AgNOR staining can be carried out on routinely processed, paraffin-embedded material, allowing simultaneous evaluation of the morphology and kinetics of the MM cells, and identifying clones of atypical plasma cells with different proliferative activity. Further studies are in progress to verify the impact of AgNOR analysis on the evolution of the disease in myeloma patients.

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References

Barlogie B, Latreille J, Alexanian R, Swartzendruber DE, Smallwood L, Maddox AM, Raber MN, Drewinko B (1982) In: Salmon SE (ed) Quantitative cytology in myeloma research. Saunders, London, pp 19–46

Barlogie B, Alexanian R, Gehan E, Smallwood L, Smith T, Drewinko B (1983) Marrow cytometry and prognosis in myeloma. J Clin Invest 72:853–861

Bartl R, Frisch B (1989) Bone marrow histology in multiple myeloma: prognostic relevance of histologic characteristics. Hematol Rev 3:87–108

Bartl R, Frisch B, Burkhardt R, Fateh-Moghadam A, Mahl G, Gierster P, Sund M, Kettner G (1982) Bone marrow histology in myeloma: its importance in diagnosis, prognosis, classification and staging. Br J Haematol 51:361–375

Bartl R, Frisch B, Burkhardt R, Jaeger K, Pappenberger R, Hoffmann-Fezer G (1984a) Lymphoproliferations in the bone marrow: identification and evolution, classification and staging. J Clin Pathol 37:233–254

Bartl R, Frisch B, Kettner G, et al. (1984b) Histologic classification of lymphoproliferative disorders in the bone marrow. Bibl Haematol 50:98–127

Bartl R, Frisch B, Burkhardt R (1985) Bone marrow biopsies revisited. A new dimension for haematologic malignancies, 2nd edn. Karger, Basel, pp 58–70

Bartl R, Frisch B, Fateh-Moghadam A, Kettner G, Jaeger K, Sommerfeld W (1987) Histologic classification and staging of multiple myeloma. Am J Clin Pathol 87:324–355

Cairns P, Suarez V, Newman J, Crocker J (1989) Nucleolar organizer regions in transitional cell tumors of the bladder. Arch Pathol Lab Med 113:1250–1252

Carter A, Hocherman I, Linn S, Cohen Y, Tatarsky I (1987) Prognostic significance of plasma cell morphology in multiple myeloma. Cancer 60:1060–1065

Committee of the Chronic Leukemia-Myeloma Task Force, National Cancer Institute (1973) Proposed guidelines for protocol studies, II. Plasma cell myeloma. Cancer Chemother Rep 4:145–158

Crocker J, Nar P (1987) Nucleolar organizer regions in lymphomas. J Pathol 151:111–118

Crocker J, Skilbeck N (1987) Nucleolar organizer regions in melanocytic lesions: a quantitative study. J Clin Pathol 40:885–889

Crocker J, Macartney JC, Smith PJ (1988) Correlation between DNA flow cytometry and nucleolar organizer regions in non-Hodgkin's lymphoma. J Pathol 154:151–156

De Gramont A, Benitez O, Smadja N, Brissaud P, Sirinelli A, Hubert D, Krulik M, Debray J (1984) Bone marrow plasma cell infiltration in multiple myeloma. Br J Haematol 57:351–352

Derenzini M, Romagnoli T, Ceccarelli C, Eusebi V (1988a) Fixatives and silver stainability of NOR proteins at the light microscopic level. J Histochem Cytochem 36:1453–1454

Derenzini M, Romagnoli T, Mingazzini P, Marinozzi V (1988b) Interphasic nucleolar organizer region distribution as a diagnostic parameter to differentiate benign from malignant epithelial tumors of human intestine. Virchows Arch [B] 54:334–340

Derenzini M, Betts CM, Trerè D, Mambelli V, Millis RR, Cancellieri A, Eusebi V (1990a) Diagnostic value of silver-stained interphase nucleolar organizer regions in breast cancer. Ultrastruct Pathol 14:233–245

Derenzini M, Pession A, Trerè D (1990b) Quantity of nucleolar silver-stained proteins is related to proliferative activity in cancer cells. Lab Invest 63:137–140

Dervan PA, Gilmartin LG, Loftus BM, Carney DN (1989) Breast carcinoma kinetics. Argyrophilic nucleolar organizer region counts correlate with Ki-67 scores. Am J Clin Pathol 92:401–407

Deschênes J, Weidner N (1990) Nucleolar organizer regions (NORs) in hyperplastic and neoplastic prostate disease. Am J Surg Pathol 14:1148–1155

Durie BMG (1984) Annotation. Is myeloma really a monoclonal disease? Br J Haematol 57:357–363

Egan M, Freeth M, Crocker J (1988a) Intraepithelial neoplasia, human papilloma virus infection and argyrophilic nucleoprotein in cervical epithelium. Histopathology 13:561–567

Egan M, Raafat F, Crocker J, Williams D (1988b) Comparative study of the degree of differentiation of neuroblastoma and mean numbers of nucleolar organizer regions. J Clin Pathol 41:527–534

Egan M, Freeth M, Crocker J (1990) Relationship between intraepithelial neoplasia of the cervix and the size and number of nucleolar organizer regions. Gynecol Oncol 36:30–33

Giri DD, Nottingham JF, Lawry J, Dundas SAC, Underwood JCE (1989) Silver-binding nucleolar organizer regions (Ag-NORs) in benign and malignant breast lesions: correlation with ploidy and growth phase by DNA flow cytometry. J Pathol 157:307–313

Hall PA, Crocker J, Watts A, Stansfeld AG (1988) A comparison of nucleolar organizer region staining and Ki-67 immunostaining in non-Hodgkin's lymphoma. Histopathology 12:373–381

Hara A, Hirayama H, Sakai N, Yamada H, Tanaka T, Mori H (1990) Correlation between nucleolar organizer region staining and Ki-67 immunostaining in human gliomas. Surg Neurol 33:320–324

Kim I, Park SH, Paik SY (1988) Nucleolar organizer regions of lymphomas in Korea. J Korean Med Sci 3:99–105

Krzyzaniak R, Buss DH, Cooper M, Wells HB (1988) Marrow fibrosis and multiple myeloma. Am J Clin Pathol 89:63–68

Latreille J, Barlogie B, Dosik G, Johnston D, Drewinko B, Alexanian R (1980) Cellular DNA content as a marker of human multiple myeloma. Blood 55:403–409

Nikicicz EP, Norback DH (1990) Argyrophilic nucleolar organizer region (AgNOR) staining in normal bone marrow cells. J Clin Pathol 43:723–727

Oken MM (1984) Multiple myeloma. Med Clin North Am 68:757–787

Ooms ECM, Veldhuizen RW (1989) Argyrophilic proteins of nucleolar organizer region in bladder tumours. Virchows Arch [A] 414:365–369

Orita T, Kajiwara K, Nishizaki T, Ikeda N, Kamizyo T, Aoki H (1990) Nucleolar organizer regions in meningioma. Neurosurgery 36:43–46

Paule B, Quillard J, Bisson M, Kahn MP, Massias P (1987) Prognostic significance of bone marrow patterns in 43 stage III multiple myeloma. Cancer J 1:416–419

Paule B, Quillard J, Bennet P, Mariette X, Bisson M (1989) Morphologic heterogeneity and plasmablastic transformation in advanced plasmacytic/plasmablastic myeloma: a study of 35 serial bone marrow biopsies in 9 patients. Nouv Rev Fr Hematol 31:203–208

Pich A, Valente G, Margaria E, Azzoni L, Tasso M, Stramignoni A (1991) Argyrophilic nucleolar organizer region counts and Ki-67 scores in human renal cell carcinoma. Pathol Res Pract 187:482–486

Plate KH, Rüschoff J, Behnke J, Mennel HD (1990) Proliferative potential of human brain tumours as assessed by nucleolar organizer regions (AgNORs) and Ki-67 immunoreactivity. Acta Neurochir (Wien) 104:103–109

Ploton D, Menager M, Jeannasson P, Himber G, Pigeon F, Achet JJ (1986) Improvement in the staining and in the visualisation of the argyrophilic proteins of the nucleolar organizer regions at the optical level. Histochem J 18:5–14

Riccardi A, Ucci G, Luoni R, Castello A, Coci A, Magrini U, Ascari E (1990) Bone marrow biopsy in monoclonal gammopathies: correlations between pathological findings and clinical data. The Cooperative Group for Study and Treatment of Multiple Myeloma. J Clin Pathol 43:469–475

Rüschoff J, Newmann K, Contractor H, Plate K, Thomas C (1990) Assessment of nucleolar organizer regions by automatic image analysis in breast cancer: correlation with DNA content, proliferation rate, receptor status and histopathological grading. J Cancer Res Clin Oncol 116:480–485

Rüschoff J, Zimmermann R, Ulshofer B (1991) Quantitation of AgNORs in urothelial cancer. Pathol Res Pract 187:759abs

Walker RA (1988) The histopathological evaluation of nucleolar organizer region proteins. Commentary. Histopathology 12:221–223