Argyrophilic nucleolar organiser region (AgNOR) staining in normal bone marrow cells

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Abstract

Fifteen normal bone marrow aspirates were stained with the argyrophilic nucleolar organiser region (AgNOR) method. The results of the specific staining of AgNORs as well as nuclear and cytoplasmic staining were analysed. A system was devised to characterise precisely the AgNORs present in the nuclei of bone marrow cells. Particular types of bone marrow cells had a characteristic AgNOR and non-AgNOR staining pattern. The bone marrow cells were identified easily and reliably with AgNOR staining and the method was especially useful for lymphocytes, plasma cells, erythroid cells, basophils/mast cells, monocytes and cells containing haemosiderin. The immature haemopoietic cells exhibited more and larger AgNORs than the more mature cells.

It is concluded that AgNOR staining can be used to study bone marrow cells by providing additional information when used in conjunction with conventional stains.

Nucleolar organiser regions (NORs) are loops of chromosomal DNA which contain clusters of ribosomal RNA genes. The silver staining method for argyrophilic nucleolar organiser region-associated proteins (AgNORs) has been used to visualise NORs in chromosomes¹ and nucleoli.² The number and size of AgNORs correlate with the level of rDNA transcription,^{3,4} the degree of cell proliferation, evidenced by the percentage of cells in S-phase,^{5,6} and the growth fraction determined by Ki-67 monoclonal antibody.⁷ The appearance of nucleoli may be useful in determining whether a cell is in interphase, or late, or early phases of cellular division.⁸

The original AgNOR method¹ has been modified^{9,10} and used to characterise malignant cells in cytological preparations¹¹⁻¹² and histological sections in a variety of tissues.¹³⁻²⁶ The AgNOR method has been applied to analyse chromosomes from normal and leukaemic bone marrow cells,²⁷ but to our knowledge its usefulness in the cytological evaluation of haemopoietic cells from bone marrow has not been assessed.

Methods

Fifteen bone marrow aspirates from the same number of patients were examined. Aspirates

were evaluated for malignancy, thrombocytopenia, and anaemia. The selected samples were cellular, showed good maturation of the three cell lines, contained normal numbers of monocytes, lymphocytes, and plasma cells, and were not malignant.

Smears dried in air were fixed in absolute methanol for five minutes, dried using a Whatman filter, washed in deionised water, and dried again. The staining method developed by Ploton *et al* was used.¹⁰ Colloidal silver staining solution was prepared by dissolving gelatine in 1% aqueous formic acid at a concentration of 2% and mixing one volume of this solution with two volumes of 50% silver nitrate. The method was slightly modified by filtering the final, freshly prepared solution (Whatman filter), placing a few drops on a clean styrofoam, and covering them with coverslip preparations of bone marrow smears. After 30 minutes of staining at room temperature under safelight conditions the coverslips were washed in deionised water, dried, and mounted. No counterstain was used. A positive control for the staining technique consisted of cytopsin preparations of cultured HL 60 promyelocytic leukaemia cells.

Smears were examined with an optical microscope under a $\times 100$ oil immersion lens at a total magnification of $\times 1000$. An ocular micrometer was used for measurements. The number of cells examined belonging to a particular type ranged from five to 150 in each case, depending on their abundance.

A system was devised to delineate AgNORs in the nuclei of bone marrow cells (fig 1). AgNORs were divided into two main groups depending on their size, structure, and shape. Group I structures included regular and irregular dots. Regular dots (dots) were subdivided into three categories depending on their size: small (1 μm in largest diameter), medium (1.5 μm), and large (2.5 μm). The internal texture of regular dots was uniform—that is, no “subdots” could be recognised. The largest diameter of regular dots was smaller than twice the smallest representative diameter. The largest diameter of irregular dots (chips) was equal to or larger than twice the smallest diameter, with the largest diameter not exceeding 3 μm . Chips were flattened, asymmetrical structures, usually made up of coalescing smaller subunits.

Group II structures, or blebs, exceeded 3 μm in size—that is, larger than group I structures—and consisted of coalesced group I structures—that is, their internal texture was

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Accepted for publication
21 May 1990

Figure 1 AgNOR structures and configurations.

not uniform. Blebs, like group I structures, were divided into regular and irregular and further subdivided into small and large. The largest diameter of regular blebs was smaller than twice their smallest diameter; the largest diameter of irregular blebs was equal to or exceeded twice the smallest diameter. Small blebs measured from 3–4 μm in largest diameter and the large blebs exceeded 4 μm .

To facilitate further description of AgNOR patterns, a system of AgNOR configurations was introduced (fig 1). A zero configuration meant the absence of AgNOR structures within the nucleus. A simple configuration described the presence of one or more structures belonging to a particular type, such as a

nucleus with only medium dots. Simple configurations were divided into simple group I and simple group II when one type of structure belonging to either one of these groups was present. When more than one type of structure belonging to either group I or group II coexisted in one cell, the configuration was designated compound and subdivided into compound group I and compound group II. A complex configuration pertained to the presence of both group I and group II structures.

We did not count the number of subdots within individual blebs or chips because our system of structures and configurations, which takes into account the size of AgNORs, obviates the need for such counts.

The AgNOR staining method also stained other structures, such as the whole nucleus (orange-brown), the cytoplasm (yellow), cytoplasmic granules and inclusions (bright-yellow to dark-brown), and haemosiderin (dark-brown to black). We considered these staining results to be important and evaluated their presence and intensity, referring to them as “non-AgNORs”. The results of staining of these structures were helpful in differentiating some cell types and rarely interfered with the interpretation of AgNORs.

The cells studied (tables 1 and 2) included the following categories: granulocytic series, erythroid series, megakaryocytes and platelets, lymphocytes and plasma cells, monocytic series and cells undergoing mitosis (not further classified).

Before regular evaluation of smears the optimal staining time was determined. This was found to be 30 minutes. Reaction times exceeding 40 minutes resulted in poor distinction of AgNORs due to overstaining of the nucleus and had the additional effect of staining granules in promyelocytes, neutrophils, and eosinophils. Staining times of less than 25 minutes produced a weak AgNOR stain.

Table 1 AgNOR staining in bone marrow cells

Cell type	Mean number of AgNORs per cell						Per cent of cells with AgNOR configuration types						Per cent of cells with AgNOR structure types											
	All AgNORs			Group I Mean (SD)	Group II Mean (SD)	Zero	Simple Group I I	Compound Group I I	Compound Group II II	Complex	Group II (All) LIB				Group I (All) LD				MD	SD	C	None		
	Group I + Group II Mean (SD)	Range	LRB								SIB	SRB	LD											
	Myeloblasts	3.5	(1.1)	2-6	1.6	(1.0)	1.9	(0.9)	0	0	7	0	6	87	100	0	23	7	94	87	71	29	19	0
Promyelocytes	3.4	(1.4)	1-6	1.6	(1.3)	1.8	(0.8)	0	0	19	5	9	67	95	0	24	9	77	72	33	28	11	24	0
Neutrophilic myelocytes	3.8	(1.2)	1-7	3.5	(1.4)	0.3	(0.7)	0	23	2	58	0	18	20	0	0	0	20	98	61	67	31	14	0
Neutrophilic metamyelocytes	3.3	(1.4)	1-6	3.3	(1.4)	0		0	74	0	26	0	0	0	0	0	0	0	100	20	48	63	5	0
Neutrophilic bands	0.6	(1.0)	0-3	0.6	(1.0)	0		60	37	0	2	0	0	0	0	0	0	0	40	0	4	39	0	60
Neutrophilic segmented	0		0	0		0		100	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	100
Young eosinophils	3.5	(1.1)	1-5	3.3	(1.3)	0.2	(0.3)	0	32	0	57	0	11	11	0	0	0	11	100	29	61	43	23	0
Eosinophils	1.4	(1.2)	0-4	1.4	(1.2)	0		22	72	0	6	0	0	5	0	0	0	0	78	0	10	73	0	22
Basophils mast cells	1.8	(1.6)	0-5	1.8	(1.6)	0.05	(0.2)	31	48	0	16	0	5	5	0	0	0	5	69	0	16	53	17	31
Pronormoblasts	3.1	(1.0)	1-4	0.6	(0.8)	2.5	(0.9)	0	0	15	0	40	45	100	63	44	15	53	45	22	25	0	0	0
Basophilic normoblasts	4.1	(1.5)	1-8	2.9	(2.2)	1.2	(1.1)	0	0	7	27	8	58	73	14	8	26	27	85	32	53	48	31	0
Poly/orthochromatophilic normoblasts	3.3	(2.0)	0-6	3.3	(2.0)	0		<1	69	0	31	0	0	0	0	0	0	0	100	0	31	90	13	<1
Erythrocytes	0		0	0		0		100	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	100
Megakaryocytes	23.9	(14.5)	0-54	22.2	(14.4)	1.7	(1.7)	9	0	0	41	0	50	50	17	<1	22	33	91	55	83	82	49	9
Platelets	0		0	0		0		100	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	100
Lymphocytes	1.1	(0.5)	1-5	1.1	(0.5)	0		0	87	0	13	0	0	0	0	0	0	0	100	93	5	12	0	0
Plasma cells	1.1	(0.3)	1-2	1.1	(0.3)	0		0	92	0	8	0	0	0	0	0	0	0	100	96	5	7	0	0
Young monocytes	3.7	(0.9)	2-5	3.2	(1.3)	0.6	(0.7)	0	10	0	44	0	46	46	0	0	28	18	100	80	63	26	16	0
Monocytes	3.6	(1.6)	0-7	3.6	(1.6)	0		2	27	0	71	0	0	0	0	0	0	0	98	30	81	58	19	2
Macrophages	1.9	(1.2)	2-5	1.7	(1.3)	0.2	(0.4)	0	44	12	38	0	5	17	0	0	0	17	87	55	34	29	10	0
Mitotic cells	0		0	0		0		100	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	100

LIB = Large irregular bleb(s); LRB = Large regular bleb(s); SIB = Small irregular bleb(s); SRB = Small regular bleb(s); LD = Large dot(s); MD = Medium dot(s); SD = Small dot(s); C = Chip(s).

Table 2 Representative patterns of AgNOR structures in bone marrow cells

Cell type	Representative patterns	
	Predominant pattern(s)	Range of representative patterns
Myeloblasts	2SRB + 1LD (14%)	1LRB + 1SRB + 1LD + 2MD, 3SRB + 1LD + 1MD, 2SRB + 1LD, 1SRB + 1SIB + 1MD + 1SD, 1SRB + 2LD
Promyelocytes	None	1LRB + 1SRB, 2LRB, 2SRB + 1LD + 1MD, 1SRB + 1C + 1SD, 1SRB
Neutrophilic myelocytes	None	3SRB + 2LD, 1SRB + 1LD + 1MD, 3LD + 3MD, 1MD + 1C + 1SD, 3SD
Neutrophilic metamyelocytes	3SD (16%), 4SD (15%)	4LD, 1LD + 1MD + 3SD, 1MD + 3SD, 2MD, 3SD
Neutrophilic bands	0 (60%), 1SD (25%)	2MD + 1SD, 1MD, 3SD, 1SD, 0
Neutrophilic segmented	NAS	
Young eosinophils	None	2SRB + 1C, 2LD + 2MD, 3MD + 2SD, 1MD + 1C + 1SD, 4SD
Eosinophils	1SD (34%)	2MD + 2SD, 2MD, 4SD, 1SD, 0
Basophils/mast cells	0 (31%)	1SRB + 1MD, 2MD + 1C, 4SD, 2SD, 0
Pronormoblasts	None	2LIB + 1SRB + 1MD, 1LIB + 2SIB, 1LIB + 2SRB, 2SRB + 2LD, 1LRB
Basophilic normoblasts	None	1LIB + 1SRB + 1LD, 1LRB + 1SRB + 2MD, 4SIB, 1SRB + 2MD + 1SD, 2MD + 2SD
Poly/ortho-chromatophilic normoblasts	3SD (20%), 4SD (15%)	1LD + 1C, 3MD + 2C + 1SD, 1MD + 4SD, 5SD, 1SD
Erythrocytes	NAS	
Megakaryocytes	None	3LIB + 2SIB + 13C, 2LIB + 3SIB + 1SRB + 10LD + 9MD + 1C + 4SD, 1SRB + 2SIB + 3LD + 8MD + 2SD, 6LD + 7MD + 1C + 40SD, 4LD + 8MD, 0
Platelets	NAS	
Lymphocytes	1LD (81%)	2LD, 1LD + 1SD, 2MD, 1MD + 1SD, 3SD
Plasma cells	1LD (88%)	2LD, 1LD + 1MD, 1LD + 1SD, 1LD, 1MD + 1SD
Young monocytes	None	1SIB + 1LD + 1MD + 1SD, 2SRB + 1C + 1MD, 1SRB + 1LD, 2LD + 2MD + 1SD, 1LD + 2MD
Monocytes	1MD + 3SD (9%), 1MD + 1C + 2SD (8%), 1LD + 1MD (7%), 4MD (7%)	3LD + 2MD + 2SD, 1LD + 2MD + 3SD, 1MD + 3SD, 4MD + 1C, 4SD, 0
Macrophages	1LD (32%)	
Mitotic cells	NAS	1SRB + 1C, 1LD + 4MD, 1LD, 3MD + 1SD, 3SD

NAS = No AgNOR structures; LIB = Large irregular bleb(s); LRB = Large regular bleb(s); SIB = Small irregular bleb(s); SRB = Small regular bleb(s); LD = Large dot(s); MD = Medium dot(s); SD = Small dot(s); C = Chip(s).

Results

Most cell types were recognised easily by the usual criteria—that is, the size and shape of the cell, the size and shape of the nucleus, and the presence and size of cytoplasmic granules. AgNOR-associated proteins, the nucleus as a whole, and the cytoplasm, were clearly visualised with AgNOR staining. The nuclei stained orange-brown and their contours were clearly outlined. The cytoplasm stained yellow (pale-yellow in most cell types) and was also well outlined. Particular cell types at different levels of maturation were recognised and information about the number, size, shape and spatial distribution of AgNORs within the cells was obtained. Within the nuclei of appropriate cells clearly defined silver stained structures were seen in all smears.

The overall results of AgNOR structures staining are summarised in tables 1 and 2. Table 1 presents numerical data obtained in the study; table 2 shows the predominant pattern(s) of AgNOR structures and a representative range of these patterns, from the most complex to the most simple. The

intensity of non-AgNOR nuclear and cytoplasmic staining is compared in table 3. Examples of AgNOR-stained bone marrow cells are depicted in fig 2.

Despite the care taken, it was difficult to eliminate the background consisting of minute dots of precipitate. These dots did not interfere with the interpretation of AgNOR stain.

Discussion

The purpose of this study was to determine the applicability of AgNOR staining in differentiating normal haemopoietic bone marrow cells and to relate the AgNOR configurations to the level of cell maturity.

The immature cell types contained more AgNORs and a higher percentage of complex configurations and group II structures than the more mature cells. With the maturation of cells there was a shift from a complex to a simple configuration. This may be exemplified by 87% myeloblasts and 67% promyelocytes with a complex group I, 58% myelocytes with a compound group I, and 74% metamyelocytes with a simple group I configuration. In erythroid series 45% pronormoblasts and 58% basophilic normoblasts contained a complex configuration; 69% poly/orthochromatophilic normoblasts possessed a simple group I configuration. The number of group II structures was much lower in the more mature cells; 100% myeloblasts and pronormoblasts manifested group II structures (blebs)—they were present in only 20% myelocytes and absent in poly/orthochromatophilic normoblasts. Neutrophilic metamyelocytes and bands disclosed no group II structures and segmented neutrophils lacked AgNORs. A similar trend was seen in monocytes and eosinophils (table 1). These observations most likely relate to the high

Table 3 Intensity of "non-AgNOR" nuclear and cytoplasmic staining (cells arranged according to increased intensity of staining)

Nuclear	Cytoplasmic*
Basophil/mast cell, dividing cell	Metamyelocyte, band, segmented, monocyte
Neutrophilic series, monocyte	Myelocyte, lymphocyte, plasma cell
Eosinophil, pronormoblast, basophilic normoblast	Myeloblast
Megakaryocyte	Promyelocyte
Lymphocyte, plasma cell, macrophage	Megakaryocyte
More mature erythroid cells	Polychromatophilic normoblast
	Pronormoblast
	Basophilic normoblast

*Excluding cytoplasmic granules, hemosiderin and phagocytic vacuoles

Figure 2 Bone marrow cells: (A) pronormoblast; (B) basophilic normoblast; (C) poly/orthochromatophilic normoblast; (D) lymphocytes; (E) plasma cell; (F) myeloblast; (G) promyelocyte; (H) neutrophilic myelocyte; (I) neutrophilic metamyelocyte and band; (J) segmented neutrophil; (K) immature eosinophil; (L) more mature eosinophil; (M) immature monocyte; (N) mature monocyte; (O) macrophage; (P) mitotic cell; (Q) basophil/mast cell; (R) part of a megakaryocyte; (S) platelets (AgNOR stains).

proliferative and transcriptional activity of the immature cells and the decline in the ability to proliferate with maturation.

A feature distinguishing immature erythroid cells (pronormoblasts and basophilic normoblasts) from other cells, was the presence of irregular blebs, particularly large irregular blebs. This permitted easy distinction of immature erythroid from immature granulocytic cells. Large irregular blebs, present in 63% of pronormoblasts and 14% of basophilic normoblasts, were not seen in myeloid cells. The only other cells possessing large irregular blebs, were megakaryocytes.

Interesting observations were made from studying AgNOR stained megakaryocytes. These cells exhibited the highest number of AgNORs, with an average of 23.9 in each cell. The number of AgNORs probably reflected the polyploid state of these cells. A small

percentage of megakaryocytes failed to show AgNORs. We believe that these are the more mature megakaryocytes. Interestingly, platelets, which are devoid of AgNORs, stained brown to dark-brown and were easily identifiable.

A very distinctive picture was obtained for lymphocytes and plasma cells. These cells, with their deeply orange-brown nucleus and pale-yellow cytoplasm, presented, for the most part, a distinct, single large dot. In a few instances the size of this large dot was approaching that of a small bleb. In most cells the large dot was located peripherally; only 1% of lymphocytes and 4% of plasma cells had a more central large dot.

In our experience the AgNOR method stained nucleoli even if they were not apparent in other preparations like the Wright's stain. This was especially evident in lymphocytes,

plasma cells, and poly/orthochromatophilic normoblasts.

Most basophils and mast cells showed a simple group I configuration with small dots as the predominant structure. About one third of these cells lacked AgNORs. Granules of basophils stained as small, light-brown to brown cytoplasmic dots, which, together with a pale-brownish nucleus, made basophils and mast cells easily recognisable. The granules did not obscure the nuclear contours or AgNORs. Further studies are required, however, to define the stages of maturation of basophils.

Because iron granules stained black, sideroblasts and siderocytes were readily recognised. Macrophages were easily identified by their cytoplasm filled with black-staining aggregates of haemosiderin and clear vacuoles. Occasionally the nucleus was obscured by haemosiderin. Dividing cells were immediately identifiable by their pale orange-brown condensed chromatin; they did not contain AgNORs. This should not be surprising considering that only cells in metaphase, anaphase, and telophase are recognisable as undergoing division and that AgNORs on acrocentric chromosomes are probably too small to be seen in routine preparations.

The intensity of cytoplasmic and non-AgNOR nuclear staining varied in different cell types and was helpful in their identification.

The granules of promyelocytes and neutrophils were smaller than those of eosinophils and lacked the ability to refract light. Thus neutrophilic promyelocytes and myelocytes could be distinguished from immature eosinophils. The yellow staining of cytoplasm in promyelocytes was deeper than that in myelocytes.

This study shows that particular types of bone marrow cells have characteristic AgNOR and non-AgNOR staining patterns. We contend that the bone marrow cells, especially lymphocytes, plasma cells, erythroid cells, basophils/mast cells, monocytes and cells containing haemosiderin are identified easily and reliably, and that AgNOR staining provides additional information when used in conjunction with conventional stains. The method should find application in evaluating normal and diseased bone marrows. We predict that the AgNOR method will help define cells types and maturation level in leukaemias.

- 1 Goodpasture C, Bloom SE. Visualisation of nucleolar organizer regions in mammalian chromosomes using silver staining. *Chromosoma* 1975;53:37-50.

- 2 Ploton D, Menager M, Adnet J-J. Simultaneous high resolution of Ag-NOR proteins and nucleoproteins in interphasic and mitotic nuclei. *Histochem J* 1984;16: 897-906.
- 3 Hofgartner FJ, Krone W, Jain K. Correlated inhibition of ribosomal RNA synthesis and silver staining by actinomycin D. *Hum Genet* 1979;47:329-33.
- 4 Morton CC, Brown JA, Holmes WM, Nance WE, Wolf B. Stain intensity of human nucleolus organizer region reflects incorporation of uridine into mature ribosomal RNA. *Exp Cell Res* 1983;145:405-13.
- 5 Field DH, Fitzgerald PH, Sin FYT. Nucleolar silver-staining patterns related to cell cycle phase and cell generation of PHA-stimulated human lymphocytes. *Cytobios* 1984;41:23-33.
- 6 Crocker J, Macartney JC, Smith PJ. Correlation between DNA flow cytometric and nucleolar organizer region data in non-Hodgkin lymphomas. *J Pathol* 1988;154:151-6.
- 7 Raymond WA, Leong AS. Nucleolar organizer regions relate to growth fractions in human breast carcinoma. *Hum Pathol* 1989;20:741-6.
- 8 Anastassova-Kristeva M. The nucleolar cycle in man. *J Cell Sci* 1977;25:103-10.
- 9 Howell WM, Black DA. Controlled silver staining of nucleolus organizer regions with a protective colloidal developer: a one-step method. *Experientia* 1980;36: 1014-5.
- 10 Ploton D, Menager M, Jeannesson P, Himber G, Pigeon F, Adnet J-J. Improvement in the staining and in the visualization of the argyrophilic proteins of the nucleolar organizer region at the optical level. *Histochem J* 1986;18:5-14.
- 11 Egan MJ, Freeth M, Crocker J. Intraepithelial neoplasia, human papilloma virus infection and argyrophilic nucleoprotein in cervical epithelium. *Histopathol* 1988;13:561-7.
- 12 Rowlands DC. Nucleolar organising regions in cervical intraepithelial neoplasia. *J Clin Pathol* 1988;41:1200-2.
- 13 Smith R, Crocker J. Evaluation of nucleolar organizer region-associated proteins in breast malignancy. *Histopathol* 1988;12:113-25.
- 14 Crocker J, Skilbeck N. Nucleolar organiser region associated proteins in cutaneous melanotic lesions: a quantitative study. *J Clin Pathol* 1987;40:885-9.
- 15 Howat AJ, Giri DD, Cotton DW, Slater DN. Nucleolar organizer regions in Spitz nevi and malignant melanomas. *Cancer* 1989;63:474-80.
- 16 Leong AS, Gilham P. Silver staining of nucleolar organizer regions in malignant melanoma and melanotic nevi. *Hum Pathol* 1989;20:257-62.
- 17 Crocker J, McGovern J. Nucleolar organiser regions in normal, cirrhotic, and carcinomatous livers. *J Clin Pathol* 1988;41:1044-8.
- 18 Crocker J, Nar P. Nucleolar organizer regions in lymphomas. *J Pathol* 1987;151:111-8.
- 19 Egan MJ, Crocker J. Nucleolar organizer regions in cutaneous tumours. *J Pathol* 1988;154:247-53.
- 20 Suarez V, Newman J, Hiley C, Crocker J, Collins M. The value of NOR numbers in neoplastic and non-neoplastic epithelium of the stomach. *Histopathol* 1989;14:61-6.
- 21 Egan M, Ramsden K, Crocker J. Diagnostic significance of mean numbers of nucleolar organizer regions in benign and malignant transitional tumours of nose. *Histopathol* 1988;13:579-81.
- 22 Morgan DW, Crocker J, Watts A, Shenoi PM. Salivary gland tumours studied by means of the AgNOR technique. *Histopathol* 1988;13:553-9.
- 23 Egan MJ, Raafat F, Crocker J, Smith K. Nucleolar organiser regions in fibrous proliferations of childhood and infantile fibrosarcoma. *J Clin Pathol* 1988;41:31-3.
- 24 Egan MJ, Raafat F, Crocker J, Smith K. Nucleolar organizer regions in small cell tumours of childhood. *J Pathol* 1987;153:275-80.
- 25 Delahunt B, Mostofi KF, Sesterhenn IA, Ribas JL, Avallone FA. Nucleolar organizer regions in seminoma and intratubular malignant germ cells. *Mod Pathol* 1990; 3:141-5.
- 26 Ayres JG, Crocker J, Skilbeck NQ. Differentiation of malignant from normal and reactive mesothelial cells by the argyrophil technique for the nucleolar organizer region associated proteins. *Thorax* 1988;43:366-70.
- 27 Reeves BR, Casey G, Harris H. Variation in the activity of nucleolar organizers in different tissues demonstrated by silver staining of human normal and leukemic cells. *Cancer Genet Cytogenet* 1982;6:223-30.