Polyploidy in non-neoplastic tissues

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Abstract

Aim—To investigate the prevalence and amount of polyploidy in fine needle aspiration specimens of the liver, urinary cytospin preparations, and cytospin preparations from pleural and peritoneal fluid.

Methods—Cells from 44 liver smears, 48 urine specimens, and 46 pleural and peritoneal aspirations were examined. After Feulgen restaining the DNA content of 100 randomly selected nuclei was determined using a TAS-plus image analysis system, combined with an automated microscope.

Results—Polyploidy was observed up to 16c in the liver, and up to 8c in urothelium and mesothelium. Sixty eight per cent of the liver aspirates contained polyploid nuclei. The rate in urothelium was 20.8% and in mesothelium 6.5%.

Conclusions—Polyploidy in the liver may be interpreted as being associated with tissue differentiation, but the findings in urothelium and mesothelium remain of unknown importance.

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Although human cells mostly have diploid chromosomes, polyploid cells with regularly dividing chromosomes are not uncommon.¹ Their occurrence stems from the irreversible transformation of an individual cell from a diploid to a polyploid state² and may arise in different cell cycle phases^{1 3 4}: in G₂/M phase

("endoreduplication"), prophase ("endomitosis"), and metaphase ("metaphase blockage"). In cases of endoreduplication the cell does not enter mitosis at all. Endomitosis has been explained by non-dissolution of the nuclear membrane, and metaphase blockage by impediment of the spindle apparatus after separation of the chromosomes.⁴ Polyploidy can be confirmed by the cytogenetic finding of four, eight, or even higher powers of the normal diploid chromosomal set which correspond to additional peaks in a DNA histogram at 4c, 8c... accompanied by some cells with the respective S phase values. Until now, however, cytogenetic analyses have almost exclusively been focused on neoplastic lesions.

Since the early 1950s polyploidy in hepatocytes, heart muscle cells, and megakaryocytes has been investigated systematically.^{1 4-6} The highest ploidy levels were 64c in liver cells,⁷ 32c in heart muscle cells,⁸ and 128c in megakaryocytes.⁹ Various amounts of polyploidy have been observed in cerebellar glia,^{10 11} neuronal tissue,¹² corneal endothelium of the eye,¹³ oral squamous epithelium affected by lichen planus,¹⁴ thyroid follicle cells,⁷ endocrine cells of the pancreas,¹⁵ endometrial cells showing an Arias-Stella reaction,¹⁶ granulosa lutein cells of the ovary,¹⁷ cytotrophoblastic cells,¹⁸ seminal vesicle epithelium,¹⁹ lymphocytes in HIV positive patients,²⁰ smooth muscle cells of arteries,²¹ and mesothelium²² (table). These data underline that polyploidy, although occurring mostly in a low percentage of cells, is quite common in non-neoplastic human tissues.

Polyploidy in benign tissues

Organ	Cell type	Condition	Method	Polyploidy up to	Remarks	Authors	
Brain	Cerebellar glia	Normal	C, 50	10/10	8c	8c nuclei 0.1%	Mann and Yates 1973 ^{10 11}
Brain	Neurons, nucleus dentatus	Normal newborn infant	S, 110	1/1	8c	8c nuclei are rare	Müller 1962 ¹²
Eye	Corneal endothelium	Normal	C, 300	21/31	8c	8c nuclei in 2 cases	Ikebe <i>et al</i> , 1984 ¹³
Oral cavity	Squamous epithelium	Lichen planus	C, 100	25/29	8c	>8c nuclei in 5 cases	Biesterfeld <i>et al</i> , 1991 ¹⁴
Thyroid gland	Follicle cells	Normal, goitre	C	51/51	128c	16c common, 128c in 1 case	Heide 1982 ⁷
Heart	Muscle cells	Normal, hypertrophy	S, 30	59/59	32c	32c nuclei 5–8%	Kompmann <i>et al</i> , 1966 ⁸
Liver	Hepatocytes	Normal	C	48/48	64c	64c nuclei in 1 case	Heide 1982 ⁷
Pancreas	Islets cells	Normal	S, 30	7/7	8c	2c:4c:8c = 75:20:5	Ehrie and Swartz 1974 ¹⁵
Uterus	Endometrial cells	Arias-Stella reaction	S, 100	5/5	16c	16c nuclei in 4 cases	Wagner and Richart 1968 ¹⁷
Ovary	Granulosa lutein cells	Pregnancy	S, 100	3/3	8c		Stangel <i>et al</i> , 1970 ¹⁸
Placenta	Cytotrophoblast	Normal	C, 250	73/80	16c		Kropff <i>et al</i> , 1993 ¹⁹
Seminal vesicle	Epithelial cells	Normal	S	/77	64c	32c common, 64c rare	Müller <i>et al</i> , 1973 ²⁰
Urinary bladder	Urothelium	Normal	C, 100	10/48	8c		Biesterfeld <i>et al</i> , 1993 (this study)
Lymph node	Lymphocytes	HIV infection	C, 100	3/8	8c	8c nuclei in 2 cases	Auffermann <i>et al</i> , 1986 ²¹
Artery	Smooth muscle cells	Normal	S, 200	9/9	32c	16c and 32c nuclei <1%	Barrett <i>et al</i> , 1983 ²²
Mesothelium	Mesothelial cells	Non-malignant diseases	C, 100	8/14	16c		Isoda and Hamamoto 1985 ²³
Blood	Megakaryocytes	Normal	C, 100	10/10	64c	64c nuclei in rare cases	Pfützer <i>et al</i> , 1983 ⁹
	Megakaryocytes	Paraneoplastic syndrome	C, 100	30/30	128c	128c nuclei in rare cases	Pfützer <i>et al</i> , 1983 ⁹

C = measurements on cytological specimens; S = measurement on sections.

Reports on polyploid conditions in known or putative precancerous lesions and in lesions of uncertain neoplastic potential have not been considered, as the occurrence of polyploidy might be the consequence of malignant transformation. This concerns, for example, squamous epithelium of the uterine cervix infected with human papillomavirus, dysplastic lesions of the vocal cord epithelium, atypical ductal hyperplasia in the breast, renal oncocytoma, pheochromocytoma, parathyroid or adrenal adenoma, and thyroid Hürthle cell oncocytoma.

In this study polyploidy was analysed in three tissue types which are often encountered in daily routine histopathological and cytopathological investigations. Urothelium, which had been shown to be polyploid in mice,^{23,24} was investigated in humans for the first time. Mesothelium was chosen as previous results had been contradictory,^{22,25,26} and liver cells were studied because they are a well known standard.

Methods

One hundred and thirty nine cytological specimens from 80 men (mean age 61 years) and 59 women (mean age 63 years) were investigated. There were 45 smears from fine needle aspiration biopsy specimens of the liver, 48 urinary cytospin preparations, and 46 cytospin preparations from pleural and peritoneal fluid.

The 93 specimens from liver and urine were fixed in alcohol and Papanicolaou stained; the 46 specimens from pleural and peritoneal fluid were air dried and stained with May-Grünwald-Giemsa. None of the specimens contained tumour cells. During a clinical follow up period of at least two years the patients showed no evidence of a malignant tumour.

The conventionally stained routine specimens were washed in xylene and restained with the Feulgen stain. The staining procedure was performed in a modified Shandon staining machine (Varistain-24) as described

in detail before.²⁷ Briefly, acid hydrolysis (4N HCl at 27.5 °C for 55 minutes) was followed by a 60 minute incubation with periodic acid Schiff at room temperature.

The DNA content of 100 randomly selected nuclei was determined using an image analysis system (TAS-plus, Leitz, Wetzlar, Germany), combined with an automated microscope.^{28,29} Only well preserved and individual nuclei were chosen; overlapping and clumped nuclei were not measured. The median DNA content of 30 epithelial cells served as an internal standard for the normal 2c value in each case.

Each measurement was represented in a DNA histogram (class width 0.25c). The DNA stemline ploidy was defined as the mean arithmetic DNA value of the nuclei with a DNA content around $\pm 12.5\%$ of the modal value. A DNA stemline at 2.0c (G_0/G_1 phase), a distinct number of nuclei with DNA values between 2c and 4c (S phase), and a small peak around 4c (G_2/M phase) represented a diploid DNA distribution. Ploidy levels between 5c and 8c were interpreted as indicators for polyploidy up to 8c, ploidy levels above 8c for polyploidy up to 16c.

Results

LIVER

In 44 of the 45 cases a diploid DNA distribution was observed (fig 1). Thirty (68.2%) had polyploid nuclei above the G_2/M level of 4c. Twenty nine biopsy specimens had single cells or small peaks around 8c. In one case one single nucleus with a DNA content of 15.5c was found. When sum histograms of three groups of patients with different ages (under 40, 40–60 and over 60 years) were compared using the Kolmogorov-Smirnov test, no significant differences were found.

One case (fig 2) showed a quite peculiar and unexpected DNA distribution with three distinct peaks at 2c, 4c, and 8c; there were more nuclei in the tetraploid region than in the diploid one. The highest value was

Figure 1 Sum histogram of DNA measurements on 44 Feulgen stained non-neoplastic fine needle aspiration specimens of the liver. Polyploidy was found in 30 cases (68.0%). Highest ploidy level: 15.5c.

Figure 2 Histogram of a DNA measurement on a fine needle aspiration specimen of the liver in a case of focal nodular hyperplasia. Occurrence of distinct peaks at 2c, 4c, 8c, 16c. Highest ploidy level, not shown in the figure: 32c.

measured at 32c. The biopsy specimen had been performed because of a sonographically diagnosed sharply delineated lesion 2 cm in diameter. Surgical treatment resulted in the histopathological diagnosis of focal nodular hyperplasia.

UROTHELIUM

A diploid DNA distribution pattern was found in all 48 cases (fig 3). Ten (20.8%) cases had single cells or small peaks around 8c. As most patients (42 of 48, or 87.5%) were older than 60, no comparison of different age groups was performed.

MESOTHELIUM

In all 46 cases a diploid DNA distribution pattern was observed (fig 4). Single nuclei between 5c and 8c were seen in only three cases (6.5%). When sum histograms of different age groups (under 40, 40–60 years, over 60 years) were compared using the Kolmogorov-Smirnov test, no significant differences in the DNA distribution patterns were found.

Discussion

With the exception of megakaryocytes, which show polyploidy under normal conditions ($\leq 64c$) and in paraneoplastic syndromes ($\leq 128c$) as an effect of cell maturation,^{9,30} polyploidy in non-neoplastic tissues is an incidental finding. It has been described in more than 20 tissue types (table). Its occurrence can be partly interpreted as a function of tissue differentiation or of functional adaptation.

POLYPLOIDY ASSOCIATED WITH TISSUE DIFFERENTIATION

A well investigated example for the association between polyploidy and tissue differentiation is the human liver. It contains only diploid nuclei in newborns; in childhood, single tetraploid nuclei occur; in adolescence tetraploid peaks may be observed, accompanied by rare octoploid nuclei. Octoploid

peaks and a few 16c nuclei have been found only in adults. These findings were correlated with increasing liver weight as a consequence of tissue growth during childhood and adolescence.³¹ In our experience no different DNA distribution patterns may be expected in adults. Similarly, in contrast to the normal hearts of newborns or young children,⁶ small polyploid peaks of less than 5% of cells at the 16c level are present in the normal hearts of adults.⁸ Smooth muscle cells in artery walls analogously reveal age dependent polyploidy up to 32c.²¹

POLYPLOIDY ASSOCIATED WITH FUNCTIONAL ADAPTATION

An association between polyploidy and the functional state of cells has been recognised since the early 1960s.³² To test this hypothesis, comparative investigations of the same cell type under normal conditions and under activation, proliferation, or other circumstances are required.

Although polyploidy is apparently normal in heart muscle cells as discussed above, its degree may be additionally increased by the functional state. Even in normal heart muscles the left ventricle has higher ploidy levels than the right ventricle, up to 16c,^{6,8} possibly as an effect of its comparatively higher blood pressure. In hypertrophic hearts polyploidy of the left ventricular myocardium may be increased up to 32c.^{6,8,33} Atrophic hearts may also reveal polyploid nuclei up to 32c, though rarely. This was reported in a case of anorexia nervosa in a 23 year old woman whose heart weight was only 165 g. Presumably atrophic hearts go through periods of deficiency, inducing DNA synthesis, as do normal hearts when they develop hypertrophy.³⁴

Tetraploid nuclei in acinar cells of the breast are more common during lactation (4–13%) than in normal conditions (1–2%).³⁵ Endometrial gland cells show polyploidy up to 16c during pregnancy, if the Arias-Stella phenomenon is present,¹⁶ but not during the normal menstrual cycle.³⁶ In lymph nodes of patients with early stage HIV infection, polyploid lymphocytes up to 8c may occur.²⁰

Figure 3 Sum histogram of DNA measurements on 48 Feulgen stained non-neoplastic urinary cytospin preparations. Polyploidy was found in 10 cases (20.8%). Highest ploidy level: 8.75c.

Figure 4 Sum histogram of DNA measurements on 46 Feulgen stained non-neoplastic cytospin preparations from pleural or peritoneal fluid. Polyploidy was found in three cases (6.5%). Highest ploidy level: 8.75c.

POLYPLOIDY OF UNKNOWN IMPORTANCE

In some tissues—for example, urothelium and mesothelium—polyploidy cannot be categorised according to tissue differentiation or functional adaptation, and its possible biological role remains unexplained.

It is well known from conventional urine cytology that human urothelium has cells with a normal nuclear:cytoplasmic ratio and enlarged, round, non-hyperchromatic nuclei. The assumption that those nuclei could be polyploid has not been investigated up to now. We found polyploid urothelial nuclei up to 8c; this has also been reported in mice in sparse numbers only, and only in about 20% of the cases.^{23 24}

Human mesothelial cells have already been investigated, with contradictory results. In a necropsy study increased ploidy levels up to 16c were reported in eight of 14 (57%) tumour negative cases.²² In another two studies, however, ploidy levels higher than 4c were not found at all in 59 cases.^{25 26} This contradiction may be partly explained by some doubtful methodological aspects in the first study: the frequent finding of non-diploid DNA stemline ploidies in 13 (93%) cases up to 3.5c indicates systematically falsely high measured DNA values. From Feulgen stained microphotographs presented in that study it seems, however, that polyploidy could have been confirmed in that study for the first time,²² but that its degree and frequency should not be finally estimated from it. In our study all 46 cases presented with a diploid DNA distribution pattern and some tetraploid nuclei. Only three (6.5%) cases had polyploid nuclei up to 8c, indicating that polyploidy in mesothelium occurs only rarely and only in a small number of cells.

In seminal vesicle epithelium the finding of polyploid nuclei up to 64c was interpreted as a function of aging,¹⁹ as the occurrence of polyploid nuclei is highly correlated with increasing age. The relevance of this hypothesis, however, is uncertain. Similar observations on heart muscle cells or liver cells,^{5 37} however, could as well be the effect of tissue differentiation or functional adaptation, as discussed above, so that the correlation with higher age may be purely coincidental. Polyploidy in corneal endothelium up to 8c,¹³ in oral squamous epithelium in cases of lichen planus,¹⁴ and in some benign tumours is also not explained by this.

IMPLICATIONS FOR INTERPRETING DNA

CYTOMETRIC DATA IN POLYPLOID TISSUES

DNA cytometric measurements are nowadays acknowledged as a supplementary procedure for grading malignancy. To avoid false positive interpretation, the effect of polyploidy on DNA cytometric data has to be taken into account, if diagnostic DNA cytometric measurements are to be performed in polyploid tissues.

In our series the DNA stemline, representing the G₀/G₁ phase of the cell population, remained constant at 2c in all 139 cases. This

means that non-diploid DNA stemlines in polyploid tissues may be interpreted as aneuploid and therefore as indicating neoplasia.

S phase fraction analysis has no practical value for diagnosis of malignancy or grading of malignancy, although in many malignant tumours the fraction is increased. Several non-malignant conditions—for example inflammation—may initiate proliferation. In our series no distinct increase in the S phase fraction was observed.

In the single cell interpretation mode of DNA cytometric measurements, the occurrence of nuclei with DNA contents above 5c has been taken as a marker of neoplasia.³⁸⁻⁴⁰ This will be of limited value in polyploid tissues as the number of those values must be increased.

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- 1 Winkelmann M, Pfitzer P, Schneider W. Significance of polyploidy in megakaryocytes and other cells in health and tumour disease. *Klin Wochenschr* 1987;65:1115-31.
- 2 Rieger R, Michaelis A, Green MM. *Glossary of genetics and cytogenetics*. Berlin: Springer, 1976:434-5.
- 3 Schwarzbacher HG. Zur Frage der Entstehung polyploider Zellen. *Verh Dtsch Anat Ges* 1968;62:206-11.
- 4 Brodsky WY, Uryvaeva IV. Cell polyploidy: its relation to tissue growth and function. *Int Rev Cytol* 1977;50:275-332.
- 5 Medvedev ZA. Age-related polyploidization of hepatocytes: the cause and possible role. *Exp Gerontol* 1986;21:277-82.
- 6 Sandritter W, Adler CP. Polyploidization of heart muscle nuclei as a prerequisite for heart growth and numerical hyperplasia in heart hypertrophy. *Rec Adv Stud Card Struct Metabol* 1978;12:115-27.
- 7 Heide W. Die Polyploidie der Zellkerne in Leber, Schilddrüse und Samenblasenepithel des Menschen während der 9. und 10. Lebensdekade und sie beeinflussende Faktoren. PhD Thesis. University of Düsseldorf, Germany, 1982:1-51.
- 8 Kompmann M, Paddags I, Sandritter W. Feulgen cytophotometric DNA determinations on human hearts. *Arch Pathol* 1966;82:303-8.
- 9 Pfitzer P, Winkelmann M, Schneider W. Polyploidiemuster von Megakaryozyten bei Patienten mit thrombotischer Paraneoplasie und Kontrollkollektive. *Verh Dtsch Ges Pathol* 1983;67:478-82.
- 10 Mann DMA, Yates PO. Polyploidy in the human nervous system. I. The DNA content of neurones and glia of the cerebellum. *J Neurol Sci* 1973;18:183-96.
- 11 Mann DMA, Yates PO. Polyploidy in the human nervous system. II. Studies of the glial cell populations of the Purkinje cell layer of the human cerebellum. *J Neurol Sci* 1973;18:197-205.
- 12 Müller HA. Cytophotometrische DNS-Messungen an Ganglienzellkernen des Nucleus dentatus beim Menschen. *Enschaffen Naturwiss* 1962;49:243.
- 13 Ikebe H, Takamatsu T, Itoi M, Fujita S. Cytofluorometric nuclear DNA determinations on human corneal endothelial cells. *Exp Eye Res* 1984;39:497-504.
- 14 Biesterfeld S, Füzesi L, Härle F, Böcking A. DNA-cytometric detection of euploid polyploidization in oral lichen planus. *Anal Quant Cytol Histol* 1991;13:7-10.
- 15 Ehrie MG, Swartz FJ. Diploid, tetraploid and octaploid beta cells in the islets of Langerhans of the normal human pancreas. *Diabetes* 1974;23:583-8.
- 16 Wagner D, Richart DM. Polyploidy in the human endometrium with the Arias-Stella reaction. *Arch Pathol* 1968;85:475-80.
- 17 Stangel JJ, Richart RM, Okagaki T, Cottral G. Nuclear DNA content of luteinized cells of the human ovary. Granulosa lutein cells of the corpus luteum during the normal menstrual cycle and pregnancy. *Am J Obstet Gynecol* 1970;108:543-9.
- 18 Kropff M, Rüberg B, Biesterfeld S, Böcking A. Euploid polyploidization in the normal human trophoblast. *Anal Quant Cytol Histol* 1993; (in press).
- 19 Müller HA, Diemer H, von Kietzell R. Polymorphe Großkerne im menschlichen Samenblasenepithel. *Virchows Arch (Cell Pathol)* 1973;12:281-4.
- 20 Auffermann W, Krüger GRF, Böcking A. DNA image cytometry in acquired immune deficiency syndrome (AIDS). *Anal Quant Cytol Histol* 1986;8:19-24.
- 21 Barrett TB, Sampson P, Owens GK, Schwartz SM, Benditt EP. Polyploid nuclei in human artery wall smooth muscle cells. *Proc Natl Acad Sci USA* 1983;80:882-5.

- 22 Isoda K, Hamamoto Y. Polyploid mesothelial cells in pleural fluid. *Acta Pathol Jpn* 1983;33:733-8.
- 23 Walker BE. Polyploidy and differentiation in the transitional epithelium of mouse urinary bladder. *Chromosoma* 1958;9:105-18.
- 24 Levi PE, Cowen DM, Cooper EH. Induction of cell proliferation in the mouse bladder by 4-ethylsulphonylnaphthalene-1-sulphonamide. *Cell Tissue Kinet* 1969;2:249-62.
- 25 Freni SC, James J, Prop FJA. Tumor diagnosis in pleural and ascitic effusions based on DNA cytophotometry. *Acta Cytol* 1971;15:154-62.
- 26 Krivinkova H, Pontén J, Blöndal T. The diagnosis of cancer from body fluids. A comparison of cytology, DNA measurements, tissue culture, scanning and transmission microscopy. *Acta Pathol Microbiol Scand* 1976;84:455-67.
- 27 Chatelain R, Willms A, Biesterfeld S, Auffermann W, Böcking A. Automated Feulgen staining with a temperature-controlled machine. *Anal Quant Cytol Histol* 1989;11:211-9.
- 28 Auffermann W, Repges R, Böcking A. Rapid diagnostic DNA cytometry with an automatic microscope and a TV image-analysis system. *Anal Quant Cytol Histol* 1984;6:179-88.
- 29 Sánchez L, Regh M, Biesterfeld S, Chatelain R, Böcking A. Performance of a TV image analysis system as microdensitometer. *Anal Quant Cytol Histol* 1990;12:279-84.
- 30 Polyploidization of megakaryocytes in normal humans, in patients with idiopathic thrombocytopenia and with pernicious anaemia. *Br J Haematol* 1971;20:489-501.
- 31 Swartz FJ. The development in the human liver of multiple deoxyribose nucleic acid (DNA) classes and their relationship to the age of the individual. *Chromosoma* 1956;8:53-72.
- 32 Lapham LW, Johnstone MA. Cytologic and cytochemical studies of neuroglia. II: the occurrence of two DNA classes among glial nuclei in the Purkinje cell layer of normal adult human cerebellar cortex. *Arch Neurol* 1963;9:194-202.
- 33 Adler CP, Friedburg H. Myocardial DNA content, ploidy level and cell number in geriatric hearts: post-mortem examinations of human myocardium in old age. *J Mol Cell Cardiol* 1986;18:39-53.
- 34 Moubayed PA, Pfitzer P. DNS-Gehalt und Zahl der Kerne in den myokardialen Zellen atrophischer Herzen. *Verh Dtsch Ges Pathol* 1975;59:338-43.
- 35 Izuo M, Okagaki T, Richart RM, Lattes R. Nuclear DNA content of acinar cells of the human breast during lactation. *Am J Clin Pathol* 1971;56:443-7.
- 36 Wagner D, Richart RM, Turner JY. DNA content of human endometrial gland cells during the menstrual cycle. *Am J Obstet Gynecol* 1968;100:90-7.
- 37 Paulini K, Sonntag W. Polyploidisierung—ein Altersphänomen? *Akt Gerontol* 1977;7:521-7.
- 38 Ploem-Zajier JJ, Beyer-Boon ME, Leyte-Veldstra L, Ploem JS. Cytofluorometric and cytophotometric DNA measurements of cervical smears stained using a new bicolor method. In: Pressman NJ, Wied GL, eds. *Automation of cancer cytology and cell image analysis*. Chicago: International Academy of Cytology, 1979:225-35.
- 39 Böcking A, Adler CP, Common HH, Hilgarth M, Granzen B, Auffermann W. Algorithm for a DNA-cytophotometric diagnosis and grading of malignancy. *Anal Quant Cytol Histol* 1984;6:1-8.
- 40 Nasiell K, Auer G, Nasiell M, Zetterberg A. Retrospective DNA analysis in cervical dysplasias as related to neoplastic progression or regression. *Anal Quant Cytol Histol* 1979;1:103-6.