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Pollen grains in human cytology

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This paper reports on pollen analyses carried out in the course of a ten-year investigation, on many thousands of cytological smears coming from various organs and systems of the human body, and prepared for diagnostic purposes. The frequency and the significance of the pollen records vary according to the specific cytological field taken into account. In the urinary sediment smears, nipple secretions, and needle aspirations the polliniferous smears are very few, and the pollen number per smear is low (max 14 pollen grains, belonging always or mostly to anemophilous species). In these cases, the pollen records evidence the airborne contamination during medical procedures, the same happens with most of cervico-vaginal smears. In some cervico-vaginal smears, the high frequency of pollen grains belonging to pharmaceutical taxa suggests that lavages with vegetable components were used by the patients before undergoing the test. In nasal, bronchial and conjunctival cytology greater amounts of polliniferous slides were recorded (in bronchial/nasal cytology also a higher number of pollen grains per smears, up to 114 – 428 respectively) and pollen spectra reflected the vegetational environment of the patients' living sites. In these cases, most pollen grains are thought to be really present on mucosae when the samples were taken. In these cytological fields pollen analysis may be useful for diagnostic purposes, above all in case of allergic pathology to detect the pollen grains causing the disease.

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Pollen grains are frequently found in human cytology. Generally, they are considered as contamination and thus are not taken into account by cytologists, less frequently they are not recognized as pollen grains but consequently been taken for other formations (atypical cells, eggs of nematodes, psammoma bodies). In the light of recently acquired data (Accorsi et al. 1981, 1982, 1984, 1985, 1986), we believe that such findings deserve greater attention, both because the slides should be scanned in the most accurate way, including the identification of all elements present in them, and because pollen grains are not human structures and it is therefore interesting to search for the causes of their presence in cytological smears.

Consequently, the magnitude and implication of this phenomenon may be assessed. This paper reports on an investigation carried out over a period of nearly ten years, the results being the achievement of a data set obtained through the analysis of a great number of cytological smears coming from various human organs and systems for diagnostic purposes. The paper has the aim of focusing the attention of cytologists on these findings and to assess the magnitude and significance of pollen presence in cytological material.

MATERIALS AND METHODS

The investigation was carried out for diagnostic purposes on 52459 cytological smears from 1980 to 1989. The slides were brought to the Institute of Pathological Anatomy, University of Modena and came from all Regional Out-Patients Departments of the province of Modena. They originated from various organs, systems or lesions of the patients living in the above mentioned province. The smears were collected in different ways: 1. from desquamation products (nasal, bronchial, cervico-vaginal, urinary, nipple, conjunctival), 2. by superficial scraping or brushing

Table I. *Results of the pollen analyses of the smears.*

NS: Nasal secretions, BA: Bronchial aspirations and brushing, BAL: Broncho-alveolar lavages, CS: Conjunctival secretions, CVS: Cervico-vaginal smears, USS: Urinary sediment smears, NPS: Nipple secretions, NA: Needle aspirations, TOT: Total smears examined.

	NS	BA	BAL	CS	CVS	USS	NPS	NA	TOT
no									
Smears examined	667	2949	489	110	40023	1996	1416	4809	52459
Polliniferous smears	160	383	10	21	81	241	7	24	962
Pollen number	3–428	1–114	1–4	1–4	1–113	1–12	1–5	1–428	
Smears with ≥ 15 pollen grains	16	23	0	0	5	0	0	0	44
%									
Polliniferous smears/ smears examined	24.0	13.0	2.0	19.1	0.2	12.1	0.5	0.5	1.8
Smears with ≤ 15 p.g./ smears examined	2.4	0.8	–	–	0.01	–	–	–	0.08
Smears with < 15 p.g./ polliniferous smears	90.0	94.0	100.0	100.0	93.8	100.0	100.0	100.0	95.4
Smears with ≥ 15 p.g./ polliniferous smears	10.0	6.0	–	–	6.2	–	–	–	4.6

(cervical scraping, bronchial brushing), 3. by lavages (broncho-alveolar lavages <BAL>), 4. by needle aspiratios of an organ, a pathologic mass, a body cavity (serous effusions, lymph node, thyroid, breast) or 5. by needle aspiration of the periphal blood. The smears were prepared taking into account the different ways of collection adopted and the features of the smears, and following the routine cytological methods. The size of the cover glasses utilized was 48 × 22 mm. The smears were stained according to the Papanicolaou methods and/or with Hematoxylin-Eosin (H&E) or with May-Grünwald-Giemsa (MGG). Precautions were taken to avoid airborne contamination. Clinical history of all patients enrolled in the study was collected. In the course of the cytological examinations 962 polliniferous smears were selected (Table I). They were submitted to pollen analysis at the Palynological Laboratory of the Experimental Evolutionistic Biology Department of the Bologna University and at the Palynological Laboratory of the Botanical University Institute in Modena. The pollen analyses were conducted using Leitz Laborlux Microscopes with 25×, 40×, 100× objectives and 10× oculars. All pollen grains present were counted and identified and their position with respect to the cytological material scanned (at the same depth of focus or at different depth of focus). In addition, note was taken if they were stained or unstained. Pollen identification was based on pollen keys, pollen atlas literature (Ciampolini & Cresti 1981, De Leonardis et al. 1987, Erdtman et al. 1961, 1963, Fœgri & Iversen 1975, Feliziani 1986, Hyde & Adams 1958, Mandrioli & Puppi 1978, Moore & Webb 1978, Nilsson et al. 1977), and fresh and acetolyzed pollen collections belonging to the two above mentioned palynological laboratories.

RESULTS

The results of the pollen analyses were interpreted taking into account the results of the cytological examinations and the clinical history. The results of the pollen analyses are given in Table I. The polliniferous slides were high in number in nasal cytology (24% of the smears were polliniferous), conjunctival (19%), bronchial (13%) and urinary (12%). In the other fields polliniferous slides were very low in number (2.0–0.2%). In Table II the pollen types recorded in the various cytological fields are reported with an indication of their frequency expressed as percentage of the total amount of pollen recorded in each cytological field. The ratio between anemophilous and entomophilous pollen grains, calculated as percentage of the total amount of pollen recorded in each cytological field, is also reported in Table II. It was observed that in all fields, except the cervico-vaginal cytology, anemophilous pollen grains are prevailing. Gramineae and Urticaceae pollen grains were most frequently recorded in all cytological fields except for the cervico-vaginal cytology in which *Matricaria chamomilla* and another pollen type, not clearly identified, but similar to the pollen grains of Hamamelidaceae (cf. *Hamamelis*), were recorded with the greatest frequency. Other

Table II: *Taxa recorded in the different cytological fields.* Percentages are calculated on the total amount of pollen recorded in each cytological field.

NS: Nasal secretions, BA: Bronchial aspirations and brushing, BAL: Broncho-alveolar lavages, CS: Conjunctival secretions, CVS: Cervico-vaginal smears, USS: Urinary sediment smears, NPS: Nipple secretions, NA: Needle aspirations. ++++: >50%, +++: 49–15%, ++: 14–5%, +: 4–1%, •: <1%.

Taxa	NS	BA	BAL	CS	CVS	USS	NPS	NA
Araliaceae		•						
Berberidaceae	•							
Betulaceae								
Alnus	•	•			•	•		
Betula	•	•				•		
Boraginaceae	•				•			
Campanulaceae	•	•						
Cannabaceae	•	•						
Caprifoliaceae	•	•						
Caryophyllaceae		•						
Chenopodiaceae	•	•				•		
Cistaceae	•	•						
Compositae								
Artemisia	•	•			•	+	++	+
Matricaria					+++			
Asteroideae		•			•	•		
Cichorioideae	•	•				•		
Convolvulaceae		•						
Cornaceae	•	•						
Corylaceae								
Carpinus	•	•				•		
Corylus	+	+	+			•		
Ostrya	++	++	++	++	+	++	++	+
Cruciferae		•						
Cupressaceae								
Cupressus	+	•	+			•		
Juniperus	+	•						
Cyperaceae	•	•						
Ericaceae		•				•		
Fagaceae								
Castanea	•	•				•		+
Fagus	•							
Quercus	++	+	+	+		++		++
Ginkgoaceae	•							
Gramineae								
wild grass	++++	++++	++++	++++	++	++++	++++	++++
Avena-Triticum	•	•						
Hordeum	+	•		+	•	•		
Hamamelidaceae cf.					+++			
Hippocastanaceae	•							
Hypolepidaceae	•							

main taxa were *Ostrya, Quercus, Plantago, Platanus, Artemisia, Corylus, Cupressus* and *Juniperus.*

DISCUSSION

For cytological work it is important to note first that the pollen presence in the smears may be attributed to two different origins: 1. the pollen grains were mixed with the cytological material when the samples were taken, as they were really present on the mucosae at the moment, 2. the pollen grains came into the smears, as contamination, during sample taking and/or slide preparation. In some fields (urinary sediment smears, nipple secretions, needle as-

Table II, cont.

Taxa	NS	BA	BAL	CS	CVS	USS	NPS	NA
Juglandaceae	•	•				•		
Labiatae	•	•						
Leguminosae	•	•				•		
Liliaceae	•							
Lythraceae		•						
Moraceae		•				•		
Oleaceae								
Fraxinus	+	•		+	•	+		
Ligustrum	•					•		
Onagraceae	•							
Palmae		•						
Papaveraceae	•	•				•		
Pinaceae								
Abies		•						
Cedrus	•					•		+
Picea					•			
Pinus	+	•		+		+		+
Plantaginaceae	++	+	+	++	•	++	++	+
Platanaceae	++	+		+	•	+		
Polygonaceae								
Polygonum	•							
Rumex	•	•				•		
Ranunculaceae	•	•				•		
Rosaceae	•	•		+	•			
Rubiaceae	•							
Salicaceae								
Populus	•	•			•	•		+
Salix	•	•			•	•		
Scrophulariaceae	•	•						
Taxaceae		•						
Taxodiaceae		•						
Tiliaceae	•	•				•		
Typhaceae	•							
Ulmaceae								
Celtis	•					•		
Ulmus	•	•				•		
Umbelliferae	•					•		
Urticaceae	+	++	+++	+++	+	++	+++	++
Vitaceae	•							
Anemophilous taxa	97.5	97.0	100	98.2	21.1	96.6	100	100
Entomophilous taxa	2.5	3.0	–	1.8	78.9	3.4	–	–
Pollen sum	5490	3531	22	56	430	1014	17	60

pirations) pollen records might be attributed only to contamination. Usually, the pollen contamination appeared to be limited to 1–5 pollen grains per smear, and were always lower than 15 pollen grains per smear. This observation is in line with the investigations recently carried out on pollen contamination in laboratories (Accorsi et al. in press). In other fields (respiratory, cervico-vaginal and probably also in conjunctival cytology) both origins were taken into account: contamination by pollen according to what has been stated for the above-mentioned fields and real pollen presence on mucosae when the

samples were taken. In this second case the pollen records may be of different significance according to the various cytological fields in which they were observed.

The palynological results obtained for the various cytological fields investigated are discussed taking into account both above mentioned origins and considering the results of the cytological examinations and the clinical history of the patients enrolled in the study.

Nasal secretions

In this field, pollen findings were recorded with the highest frequency (24% of smears). In most cases pollen grains were low in number (3–12 per smear), in some cases (10% of the polliniferous smears) the smears display a pollen content higher than 15 grains per slide (from 22 to 428 with various cases in which the pollen content was higher than 100 grains per slide). The pollen grains were mainly airborne. They were at the same depth of focus of the cytological material, clearly included in it, and stained. Pollen grains at higher depth of focus occurred less frequently and unstained pollen grains both clearly evidence for contamination of the cytological slides – were rarely found. Pollen spectra generally reflected the aeropalynological calendars drawn up by the Prevention's Department of Modena and referring to the time at which the samples were taken and the slides were prepared. The differences found were due to the various vegetational environment of the patients' living sites. The highest pollen content was recorded in April and May. The smears with low pollen content came both from patients with vasomotory rhinitis and from those suffering from allergic rhinopathies, the latter being either pollinosic or nonpollinosic. The smears showing a pollen content higher than 15 grains per slide were recorded in pollinosic patients with cytological inflammatory processes; in the smears of these patients the presence of a high number of eosinophils granulocytes demonstrated the existence of allergic rhinopathies.

As far as the pollen source and the significance of pollen presence in the nasal secretions is concerned, two possible sources were involved. We weren't able, however, to quantify the two different amounts. We are of the opinion that the presence of pollen grains in few cases was due to airborne pollution, and that the majority of them was really present on the nasal mucosa when the secretions were taken. Moreover, in the case of pollinosis patients, it emerged that the pollen analysis of the nasal secretions emitted during rhinorrhea may be of help for diagnostic purposes, as it shows the spectrum of allergenic pollens inhaled at the moment of the attack, and enables skin tests and total IgE level and specific IgE level testing to be carried out in a more specific way.

Bronchial aspirations

Pollen grains were recorded in 13% of smears. In most cases, pollen grains were low in number (1–14 per smear); in some cases (6% of the polliniferous smears) the smears display a pollen content higher than 15 grains per slide (from 16 to 114, with various cases in which the pollen content was higher than 40 grains/slide). As for the pollen location with respect to the cytological material scanned and as for the number of pollen grains stained, the observations gave similar results to those recorded for nasal secretions. The pollen spectra also, were similar. The smears containing less than 15 pollen grains per slide were observed both in patients showing a variety of pathologies (bronchial inflammations in some cases associated with epidermoid metaplasia, lung neoplasm, viral inflammations) and in patients without pathology. The smears with more than 15 pollen grains were observed in patients sufferring from bronchial inflammations associated with epidermoid metaplasia. Also in bronchial cytology as well as in nasal cytology we supposed two different pollen sources: a portion might come from contamination and the other part might be truly present on the bronchial mucosae (Martonen & O'Rourke unpubl., Melillo & Scala 1986, Perino et al. 1986) and, thus, in the cytological material when the samples were taken. According to our survey the above situation presumably takes place at the onset of pathological conditions, when the efficiency of the vibratile epithelium, having the function of conveying the foreign particles to the outside, is lowered.

Broncho-alveolar lavages (BAL)

Pollen grains were recorded in 2.0% of smears (1–4 per smear). They were therefore infrequent and low in number. The pollen grains were both at the same depth of focus and at different depth of focus with respect to the cytological material; in some cases they were unstained. They belonged exclusively to anemophilous species. A part of the pollen records

was probably due to contamination, and the other part might be considered present in the lungs when the lavage was performed (Dankaart et al. unpubl., Melillo & Scala 1986, Perino et al. 1986).

Urinary sediment smears

Pollen grains were recorded in 12% of smears. They were, therefore, rather frequent, but the number of pollen grains per smear was low (1–8, max. 12). Pollen grains were both at the same depth of focus with respect to the cytological material and at different depth; in comparison with the above mentioned cytological fields; the unstained pollen grains were more frequent, this being a clear evidence of contamination. The pollen grains belonged mostly to anemophilous species. The presence of pollen records might be attributed to contamination, which in this field may occur more often as urine samples are taken by the patients themselves, without particular precautions to avoid air contaminations.

Conjunctival secretions

Pollen grains were recorded in 19% of cases (1–4 per slide); they were thus rather frequent, but very low in number. They were mainly airborne, at the same depth of focus of the cytological material and all stained. The pollen grains were found in the smears of patients suffering from conjunctivitis of various origin; they were recorded most frequently, however, in case of allergic conjunctivitis. The interpretation of the pollen presence on the basis of the actual state of our survey seems to be rather difficult, but, owing to the pollen record frequency, to the absence of unstained pollen grains and to the preparation technique to avoid pollen contamination, the pollen presence may not be considered only as a consequence of contamination phenomena. Anyway, the identification of pollen grains correlated with a clear cytological evidence of allergic syndromes, might supply further details useful to the identification of the allergens.

Cervico-vaginal smears

Pollen grains were recorded in 0.2% of cases. They had therefore to be considered as infrequent. In most cases they were also low in number (1–4, seldom 8 per smear). Pollen grains were both at the same depth of focus with respect to the cytological material and at different depth of focus; sometimes they were unstained, nearly all were anemophilous. Their presence might not be correlated with particular clinical history. In our opinion, in these cases, the pollen records might be attributed to the contamination of the cytological material. The exception to this survey was represented by five smears, in which a high number of pollen grains was recorded (70–113 per slide); they belonged almost exclusively to *Matricaria chamomilla* or to another pollen type not clearly identified, but similar to Hamamelidaceae (cf. *Hamamelis*). They were all stained, nearly all at the same depth of focus of the cytological material and clearly incorporated in it. The clinical history of the patients suggested a correlation of the pollen records to the use of lavages containing vegetal components shortly before the samples were taken.

Nipple secretions and needle aspirations

Pollen grains were recorded in 0.5% of cases (1–5 per slide). They were therefore infrequent and low in number. They were found both at the same depth of focus with respect to the cytological material and at different depth of focus. Sometimes they were found to be unstained. All pollen grains belonged to anemophilous taxa and were from cytological material prepared throughout the year. We believe that the pollen records had to be referred exclusively to contamination of the smears. This inference confirms the hypothesis according to which pollen grains should not be found in cytological material coming from inner organs with no direct communication to the outside.

CONCLUSIONS

The results obtained during the survey led us to the following conclusions: smears containing pollen grains account on average for the 1.8% of all cytological smears investigated, with percentages ranging from 0.2 to 24% according to the cytological field taken into account. They may be considered as a recurrent presence about which cytologists should be informed to reach targeted diagnoses and to avoid mistakes. In all cytological fields a part of the pollen records was probably due to the contamination occurring when the samples were taken or during the preparation of the slides.

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