Virchows Arch (2000) 437:198–202

CASE REPORT

Silvia Cristina · Renzo Boldorini · Francesco Brustia
Guido Monga

Lymphoepithelioma-like carcinoma of the breast. An unusual pattern of infiltrating lobular carcinoma

Received: 19 August 1999 / Accepted: 3 February 2000

Abstract A case of breast carcinoma, showing both lymphoepithelioma-like and lobular infiltrating carcinoma, is described, which must be distinguished from the medullary carcinoma with which it shares some features, such as the strong lymphocytic infiltration, but not sharp circumscription, syncytial growth pattern, nuclear pleomorphism, and high mitotic rate. Unlike the lymphoepithelial carcinoma of the nasopharynx and some lymphoepithelioma-like carcinomas of the lung, stomach, salivary glands, and thymus, it does not seem to be connected with Epstein-Barr virus (EBV) infection, as shown by negative results of both in situ hybridization and polymerase chain reaction. This neoplasia may be defined as a peculiar form of lobular carcinoma, therefore, more representative of an unusual microscopic pattern than a distinctive clinicopathologic entity in itself.

Key words Lymphoepithelioma-like carcinoma · Breast · EBV · Immunostaining

Introduction

In 1994, Kumar et al. [15] reported a case of primary lymphoepithelioma-like carcinoma of the breast (LELC-B), which was defined as a distinctive primary malignant neoplasm with striking microscopic similarity to undifferentiated carcinoma (lymphoepithelioma) of the nasopharynx [18]. It shares histologic features with other LELCs, which, in recent years, have been seen in many other sites, including the skin [30], larynx [1], thyroid [29], salivary glands, stomach [19], lungs [2], thymus, uterine cervix [31], prostate, and urinary tract [24].

As in the lymphoepithelioma of the nasopharynx, Epstein-Barr virus (EBV) has been detected in some LELCs from other sites. In a recent review of the literature on LELC, Iezzoni et al. [12] found EBV consistently associated with four anatomic sites – lung [2, 22], stomach [34], salivary gland [10], and thymus [33]. In the previous reported case of LELC-B [15], the EBV genome was not detected by in situ hybridization but, as claimed by the authors, this could be due to the low sensitivity of the method; nevertheless, EBV was also absent in medullary breast carcinomas, which share many characteristics with LELCs, by both in situ hybridization and polymerase chain reaction (PCR) [16].

To the best of our knowledge, herein we report the second case of LELC-B with particular emphasis on its histologic features, criteria of differential diagnosis with medullary carcinoma (MC), and the results of EBV detection using both in situ hybridization and PCR.

Clinical history

A 54-year-old female was noted to have a palpable nodule in the upper outer quadrant of the right breast on clinical examination; mammography confirmed the presence of a well-circumscribed mass of 1.5 cm in diameter, located deep within the breast tissue. A quadrantectomy was performed. On close inspection, a nodule with pushing margins was found near the lateral surgical margin. A diagnosis of infiltrating carcinoma, probably of the medullary type, was made on frozen sections. The patient underwent a more extensive excision of the breast and axillary node dissection. After a definitive diagnosis, a course of radiotherapy was carried out. Six months after the initial procedure, the patient was well without evidence of recurrence.

Materials and methods

Several samples from the neoplastic nodule and the surrounding mammary tissue were formalin fixed, paraffin embedded, and investigated by means of histology, immunohistochemistry, in situ

S. Cristina (✉) · R. Boldorini · G. Monga
Dipartimento di Scienze Mediche, Facoltà di Medicina
e Chirurgia, Università del Piemonte Orientale
„Amedeo Avogadro“, Servizio di Anatomia Patologica, Ospedale
Maggiore della Carità, Corso Mazzini 18, I-28100 Novara, Italy
Tel.: +39-321-3733932, Fax: +39-321-3733485

F. Brustia
Dipartimento di Scienze Mediche, Facoltà di Medicina
e Chirurgia, Università del Piemonte Orientale
„Amedeo Avogadro“, Clinica Chirurgica, Novara, Italy

hybridization, and PCR for the EBV genome. For histologic examination, 4-µm-thick paraffin sections were stained with haematoxylin and eosin.

Immunohistochemistry

The streptavidin–biotin peroxidase technique was employed for immunohistochemical stains using the following monoclonal antibodies: cytokeratin (CK; Biomeda, Foster City, Calif.; prediluted); EMA (NeoMarkers, Union City, Calif.; prediluted); CD45 (BioGenex, San Ramon, Calif.; prediluted); CD20 (Dako, Denmark; dilution 1:80); CD45RO (Dako; dilution 1:80); estrogen and progesterone receptors (Immunotech, Marseille, France; dilution 1:50); pS2 (BioGenex; dilution 1:100); p53 (BioGenex; dilution 1:150); c-erb-B2 (BioGenex; prediluted); MIB-1 (Immunotech; dilution 1:100), and e-cadherin (BD Tranduction Laboratories, Lexington, Ky.; dilution:1:1000). The following tissues were used as positive and negative (omitting the primary antibody) controls: human tonsil for CD45, CD45RO, CD20, and MIB-1; peripheral areas of non-neoplastic mammary tissue as well as two cases of infiltrating lobular and ductal mammary carcinoma for CK, EMA, estrogen, and progesterone receptors, pS2, p53, c-erb-B2, and e-cadherin. In the evaluation of hormone-receptor status and c-erb-B2, the cut-off values proposed by Querzoli et al. [23] were used; *p53* is considered positive when more than 5% of the tumor cells showed a definite nuclear staining. Tumor cells were considered to be positive for MIB-1 when there was any staining of the nucleoplasm or nucleoli, regardless of staining intensity. The MIB-1 index was expressed as the percentage of positive tumor cells with nuclear staining – evaluated by counting at least 500 tumor cells in selected fields – on the total number of neoplastic cells scored. The 13% of MIB-1 index was used as a cut-off value to separate cases with low and high proliferative activities [23].

In situ hybridization

In situ hybridization was performed using a fluorescein-labeled PNA probe for short EBV RNAs (EBER, Dako, Milan, Italy), with overnight incubation after proteinase K pretreatment. A double indirect immunoperoxidase reaction with an anti-fluorescein monoclonal antibody was then used to reveal the reaction with diaminobenzidine (DAB) as chromogen. As a control, sections of primary brain B-cell lymphoma from a human immunodeficiency virus (HIV)-positive patient were used, as previously described [6].

Polymerase chain reaction

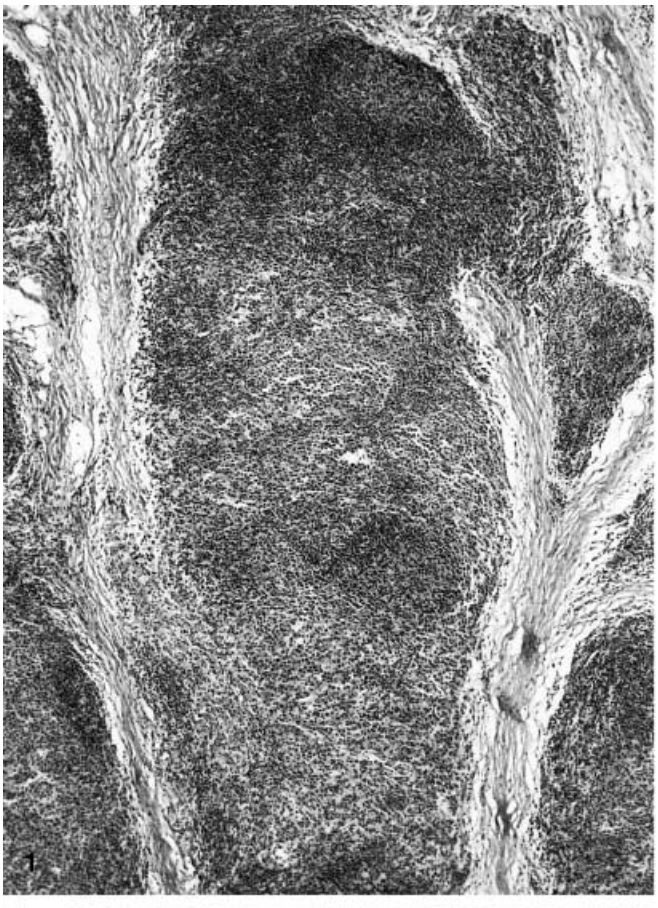
All oligonucleotides utilized in this study were synthesized using the solid-phase triester method. Analysis of EBV infection was assessed by means of single-step PCR analysis with primers representative of the EBV nuclear antigen (EBNA)-1 gene (SL-1, 5'-GGACCTCAAAGAAGAGGGGG-3'; SL-3, 5'-GCTCCTGGTCT TCCGCCTCC-3') and of the EBNA-3A gene (3A/S, 5'-GAAA CCAAGACCAGAGGTCC-3'; 3A/AS, 5'-TCCCAGGGCCGGA CAATAGG-3'). Control amplification was performed using primers derived from the p53 exon-6 sequence (P6-5, 5'-ACAGG GCTGGTTGCCCAGGGT-3'; P6-3, 5'-AGTTGAAAACCAGAC CTCAG-3') [9]. PCR was performed using 600 ng genomic DNA, 1 µM each primer, 200 µM each dNTP, 10 mM Tris-HCl (pH 8.8), 50 mM KCl, 1 mM $MgCl_2$, 0.01% gelatin, and 1 U *Taq* DNA polymerase (Perkin Elmer, Norwalk, Conn.) in a final volume of 25 µl. PCR conditions for both genes were as follows: 94°C for 2 min, followed by 35 cycles at 94°C for 40 s, 55°C for 40 s, and 72°C for 1 min. The reaction (10 µl) was visualized by means of ethidium bromide staining on a 2% agarose gel.

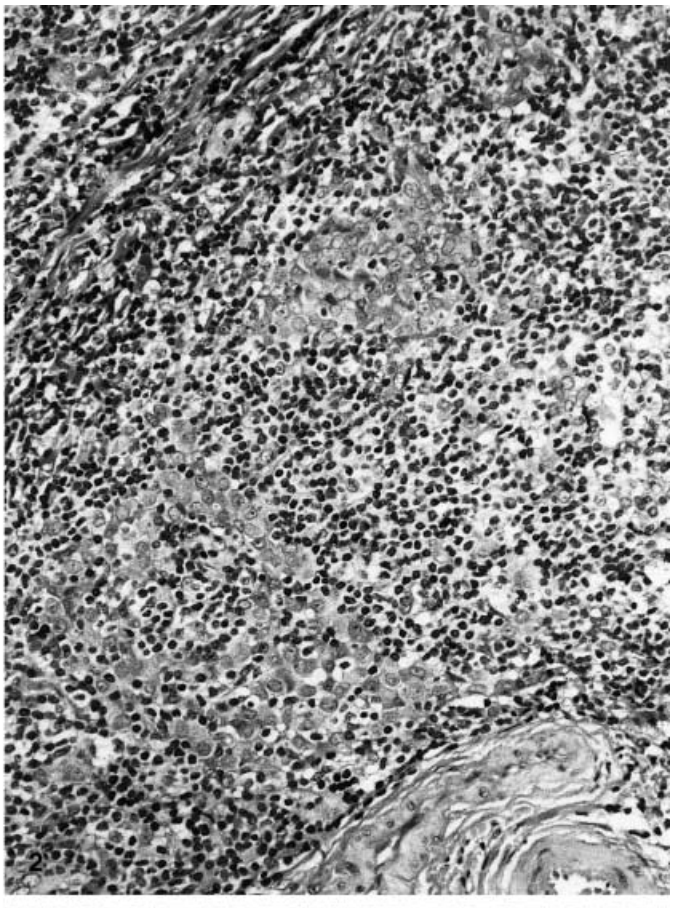
Results

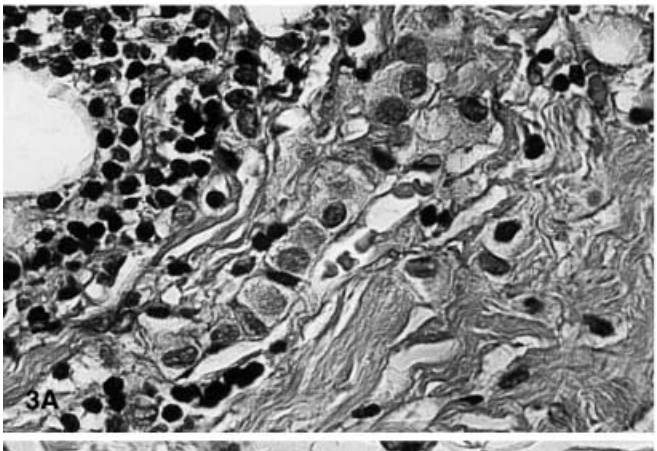
At histological examination, the tumor was mainly composed of nodular aggregates of predominantly small lymphocytes interspersed among dense collagenous stroma and fat (Fig. 1). Germinal centers were observed in a few lymphocytic aggregates. In this background, large epithelial cells with pale eosinophilic cytoplasm and regular nuclei with inconspicuous nucleoli were scattered in small clusters and isolated in the lymphocytic infiltrate (Fig. 2). No intracytoplasmic vacuoles were noted; mitosis were extremely rare. There was no evidence of a syncytial pattern of growth. In addition, between the nodular lymphoid aggregates, and at their periphery, small areas of infiltrating lobular carcinoma with a typical Indian file pattern of growth (Fig. 3A) and intraductular pagetoid spread were present; only a few foci of in situ lobular carcinoma were identified. The cytologic characteristic of the epithelial elements present in the lymphoid nodules and the cells of infiltrating lobular carcinoma were similar. No areas of necrosis were identified. No metastases were found in the 19 isolated axillary lymph nodes.

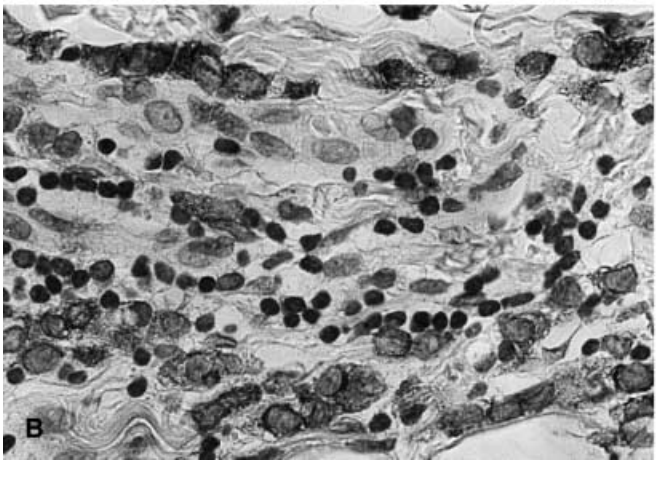
On immunostaining, the lymphocytic infiltrate was intensely stained using CD45 (leucocyte common antigen, LCA) and, using subset markers, a mixture of B and T lymphocytes was found. B- and T-cell populations did not show a clear predominance, but displayed a different distribution. Indeed, CD20 staining demonstrated B cells mainly present in the center of the nodular aggregates and the germinal centers, whereas T cells (demonstrated by CD45RO staining) were diffusely distributed. Rare aggregates of B lymphocytes were present also around a few non-neoplastic ductular structures. CK and epithelial membrane antigen (EMA) highlighted the infiltrative growth pattern in the fibrous stroma and fat (Fig. 3B) and the presence of neoplastic cells in the lymphoid nodules (Fig. 4); both details were, in part, obscured by the dense lymphocytic infiltrate in the routinely stained sections. Moreover, the CK stain revealed the presence of some non-neoplastic ductules at the center of lymphocytic aggregates, without evidence of lymphoepithelial lesions. E-cadherin immunoreactivity, clearly evident in non-neoplastic mammary tissue, was absent in neoplastic cells in the lymphoid nodules as well as in the peripheral infiltrating carcinoma. The MIB-1 index of epithelial cells was 5%. Elevated MIB-1 positivity was found in germinal centers and in lymphocytic infiltrate. The neoplastic cells were positive for estrogen (42%) and negative for progesterone (<10%) receptors. No expression of c-erb-B2/neu protein or *p53* tumor protein was found.

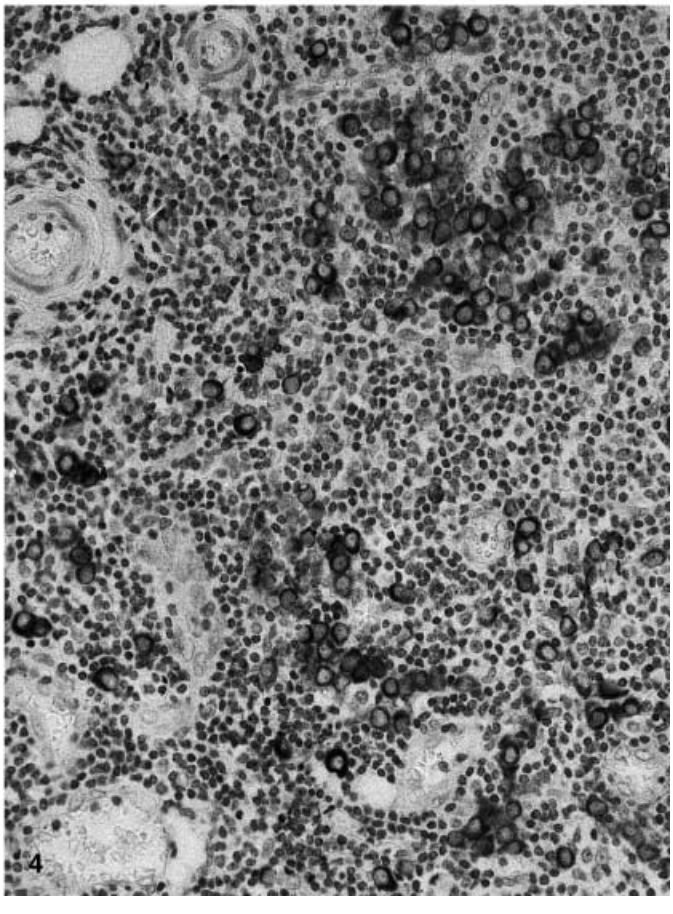
In situ hybridization and PCR for detection of the EBV genome were negative in both epithelial and lymphocytic populations. The final diagnosis of lymphoepithelioma-like infiltrating lobular carcinoma of the breast was made.


1


2


3A


B


4

Discussion

The term LELC describes tumors occurring outside the nasopharynx, which show similar morphologic features to those of undifferentiated nasopharyngeal carcinomas. Indeed, LELCs are characterized by either cohesive nests or by a diffuse growth of malignant epithelial cells in a background of inflammatory cells that are predominantly lymphocytes. Because of their undifferentiated appearance and the presence of intensely inflammed stroma widely separating the malignant cells and to some extent obscuring their presence, LELCs could be confused with lymphoproliferative lesions. Indeed, the differential diagnosis of pseudolymphoma or lymphoma of the breast was taken into account by Kumar et al. [15]. This could be also the case for our patient, but a careful examination of cytologic details, the presence of small foci of infiltrating lobular carcinoma and, in particular, the results of immunostaining for CK and EMA help us to rule out such a diagnosis.

When histologic criteria of LELCs are applied to breast neoplasias, it is not surprising that some authors regard MC as the breast counterpart of LELC [16]. Histologic recognition of MCs, a subgroup of infiltrating ductal carcinomas with a more favorable prognosis, is based on the following criteria, as proposed by Fisher [8] and Ridolfi [27]: (1) a predominantly syncytial growth pattern (>75%), (2) sparse stroma, (3) microscopically completely circumscribed, (4) moderate to marked mononuclear stromal infiltrate, (5) moderate to marked nuclear pleomorphism and numerous mitoses, (6) no intraductal component and (7) absence of glandular features. Moreover, Ridolfi et al. [27] identified a subgroup of MCs termed atypical (AMC), which fulfill criteria 1 and 2, plus 2–3 of the other criteria. Further studies demonstrated the clinical inconsistency of the latter category [28, 32] because these AMCs have the same outcome as infiltrating ductal carcinomas [14, 25]. Therefore, a new simplified histopathologic definition of MC was proposed by Pedersen et al. [20, 21], according the following criteria: (1) syncytial growth pattern and no tubular component, (2) diffuse stromal mononuclear infiltration, and (3) sparse necrosis (<25%).

It is evident that the tumor we are dealing with shows different histologic features, consisting, as it does, mainly of a peripheral pattern of infiltrating lobular carcinoma, the absence of a syncytial growth pattern, and necrosis. Therefore, according to the above-reported criteria, it cannot be defined as MC. This statement is further supported by the immunohistochemical results: in fact, the typical characteristics of MC, i.e., high mitotic rate, high MIB-1 index, and high incidence of intense *p53* positivity [13], were absent in our case. Moreover, as to the hormonal status, our case is estrogen-receptor positive, whereas more than 90% of MCs are immunohistochemically estrogen-receptor negative [26]. Also, lack of expression of e-cadherin is against the diagnosis of MCs: indeed e-cadherin immunoreactivity was shown to be positive in MC, as recently demonstrated [4], and in ductal carcinoma, but negative in lobular carcinoma [7]. Therefore, it seems evident that this tumor and that reported by Kumar et al. [15] represent an unusual type of infiltrating carcinoma of the breast which, because of its peculiar morphologic characteristic, can be defined as a LELC-B. The definition of LELC-B should be employed only to indicate this subtype of breast carcinoma and should not be used as an alternative term to indicate MCs. Making the distinction between LELC-B and MC is not a merely speculative exercise but, because of the better prognosis of MC, it is of practical importance for patient management. Due to the short follow-up of our case, we have no direct data as to prognosis, although the evidence of an infiltrating lobular carcinoma does not lead to an optimistic hypothesis.

The EBV genome within the tumor was not detected by either in situ hybridization or PCR, despite the similarities between our case and the LELCs of other sites such as the stomach, salivary gland, lung, and thymus, where the EBV genome was definitely identified [12]. It is noteworthy that the EBV genome has never been found in breast carcinomas [11, 16], similar to that which occurs in LELCs of other non-foregut derived organs, such as the skin and uterine cervix [3, 17].

Although lymphoid cell infiltration is fairly common in several types of breast carcinomas, the finding of lymphoid aggregates, obscuring the neoplastic epithelial cell component, as observed in our case, is unusual and intriguing. The lobulocentricity of the lymphoid infiltrates is reminiscent of the previous case of lymphocytic mastopathy associated with infiltrating lobular carcinoma reported by Chetty et al. [5], but its histologic description is quite different. First, we never observed lymphoepithelial lesions; moreover, in our case, the inflammatory infiltrate was confined to the tumor without any evidence of lymphocytic mastopathy in the adjacent breast parenchyma. Although we have excluded an EBV-linked etiology, the possibility remains that LELC-B, as is true of LELCs developing in non-foregut derived tissues, is induced by an as yet unidentified injurious or infectious entity [3]. Moreover, it cannot be excluded that such a finding could be connected to an infiltrating carcinoma that may act as the immunological trigger for the development of the lymphoid stroma.

In summary, we have reported a second case of LELC-B, a tumor with characteristic histologic findings,

◀ **Fig. 1** Low-power view of the lesion showing large nodular aggregates of lymphocytes, some with pale germinal centers. Haematoxylin and eosin, ×40

Fig. 2 Higher magnification of a nodule with small clusters of epithelial cells larger than surrounding small lymphocytes; the epithelial cells show uniform nuclei and small, inconspicuous nucleoli. Haematoxylin and eosin, ×250

Fig. 3 **A** Indian file pattern of infiltrating lobular carcinoma adjacent to lymphoid nodule; haematoxylin and eosin, ×400. **B** This pattern is highlighted by cytokeratin immunostaining; streptavidin-biotin-peroxidase, ×400

Fig. 4 Immunohistochemical staining for cytokeratin highlights the presence of scattered neoplastic epithelial cells in the lymphoid nodule, ×250

which does not appear to be linked to EBV etiology. Our observations suggest that a lymphoepitheliomatous histology is a rare, small part of the repertoire of breast lobular carcinomas and, therefore, we conclude that LELC-B could represent more an unusual microscopic pattern than a distinctive clinicopathologic entity in itself.

Acknowledgements The authors are grateful to Professor Luca Vago and Professor Gianluca Gaidano for performing in situ hybridization techniques and PCR, and to Mrs. Barbara Wade for having reviewed the English text.

References

1. Andryk J, Freije JE, Schultz CJ, Campbell BH, Komorowski RA (1996) Lymphoepithelioma of the larynx. Am J Otolaryngol 17:61–63
2. Butler AE, Colby TV, Weiss LM, Lombard C (1989) Lymphoepithelioma-like carcinoma of the lung. Am J Surg Pathol 13:632–639
3. Carr KA, Bulengo-Ransby SM, Weiss LM, Nickoloff BJ (1992) Lymphoepithelioma-like carcinoma of the skin: a case report with immunophenotypic analysis and in situ hybridization for Epstein-Barr viral genome. Am J Surg Pathol 16:909–913
4. Charpin C, Bonnier P, Garcia S, Andrac L, Crebassa B, Dorel M, Lavaut MN, Allasia C (1999) E-cadherin and beta-catenin expression in breast medullary carcinoma. Int J Oncol 15:285–292
5. Chetty R, Butler AE (1993) Lymphocytic mastopathy associated with infiltrating lobular breast carcinoma. J Clin Pathol 46:376–377
6. Cinque P, Vago L, Dahl H, Brytting M, Terreni MR, Fornara C, Racca S, Castagna A, Monforte AD, Wahren B, Lazzarin A, Linde A (1996) Polymerase chain reaction on cerebrospinal fluid for diagnosis of virus-associated opportunistic diseases of the central nervous system in HIV-infected patients. AIDS 10:951–958
7. De Leeuw WJ, Berx G, Vos CB, Peterse JL, Van de Vijver MJ, Litvinov S, Van Roy F, Cornelisse CJ, Cleton-Jansen AM (1997) Simultaneous loss of E-cadherin and catenins in invasive lobular breast cancer and lobular carcinoma in situ. J Pathol 183:404–411
8. Fisher ER, Gregorio RM, Fisher B (1975) The pathology of invasive breast cancer. Cancer 36:1–84
9. Gaidano G, Ballerini P, Gong JZ, Inghirami G, Neri A, Newcomb EW, Magrath IT, Knowles DM, Dalla-Favera R (1991) p53 mutations in human lymphoid malignancies: association with Burkitt's lymphoma and chronic lymphocytic leukemia. Proc Natl Acad Sci U S A 88:5413–5417
10. Hamilton-Dutoit SJ, Therkildsen MH, Nielsen NH, Jensen H, Hansen JP, Pallesen G (1991) Undifferentiated carcinoma of the salivary gland in Greenlandic Eskimos: demonstration of Epstein-Barr virus DNA by in situ nucleic acid hybridization. Hum Pathol 22:811–815
11. Horiuchi K, Mishima K, Ohsawa M, Aozasa K (1994) Carcinoma of the stomach and breast with lymphoid stroma: localisation of Epstein-Barr virus. J Clin Pathol 47:538–540
12. Iezzoni JC, Gaffey MJ, Weiss LM (1995) The role of Epstein-Barr Virus in lymphoepithelioma-like carcinomas. Am J Clin Pathol 103:308–315
13. Jensen V, Jensen ML, Kiaer H, Andersen J, Melsen F (1997) MIB-1 expression in breast carcinoma with medullary features. An immunohistological study including correlations with p53 and bcl-2. Virchows Arch 431:125–130
14. Jensen ML, Kiaer H, Andersen J, Jensen V, Melsen F (1997) Prognostic comparison of three classifications for medullary carcinomas of the breast. Histopathology 30:523–532
15. Kumar S, Kumar D (1994) Lymphoepithelioma-like carcinoma of the breast. Mod Pathol 71:129–131
16. Lespagnard L, Cochaux P, Larsimont D, Degeyter M, Velu T, Heimann R (1995) Absence of Epstein-Barr virus in medullary carcinoma of the breast as demonstrated by immunophenotyping, in situ hybridization and polymerase chain reaction. Am J Clin Pathol 103:449–452
17. Matias-Guiu X, Komminoth P, Prat J (1994) Absence of Epstein-Barr virus DNA in lymphoepithelioma-like carcinoma of the uterine cervix. Am J Clin Pathol 101:117
18. Micheau C (1986) What's new in histological classification and recognition of nasopharyngeal carcinoma (NPC). Pathol Res Pract 181:249–253
19. Min KW, Holmquist S, Peiper SC, O'Leary TJ (1991) Poorly differentiated adenocarcinoma with lymphoid stroma (lymphoepithelioma-like carcinomas) of the stomach. Report of three cases with Epstein-Barr virus genome demonstrated by the polymerase chain reaction. Am J Clin Pathol 96: 219–227
20. Pedersen L (1997) Medullary carcinoma of the breast. APMIS 75[suppl]:105:5–31
21. Pedersen L, Zedeler K, Holck S, Schiodt T, Muoridsen HT (1991) Medullary carcinoma of the breast, proposal for a new simplified histopathological definition. Br J Cancer 63:591–595
22. Pittaluga S, Wong MP, Chung LP, Loke S-L (1993) Clonal Epstein Barr virus in lymphoepithelioma-like carcinoma of the lung. Am J Surg Pathol 17:678–682
23. Querzoli P, Ferretti S, Albonico G, Magri E, Scapoli D, Indelli M, Nenci I (1995) Application of quantitative analysis to biologic profile evaluation in breast cancer. Cancer 76:2510–2517
24. Randolph TL, Amin MB, Ro JY, Ayala AG (1997) Histologic variants of adenocarcinoma and other carcinomas of prostate: pathologic criteria and clinical significance. Mod Pathol 10:612–629
25. Rapin V, Contesso G, Mouriesse H, Bertin F, Lacombe MJ, Piekarski JD, Travagli JP, Gadenne C, Friedman S (1988) Medullary breast carcinoma. A reevaluation of 95 cases of breast cancer with inflammatory stroma. Cancer 61: 2503–2510
26. Reiner A, Reiner G, Spona J, Schemper M, Holzner JH (1988) Histopathologic characterization of human breast cancer in correlation with estrogen receptor status. A comparison of immunocytochemical and biochemical analysis. Cancer 61:1149–1154
27. Ridolfi RL, Rosen PP, Port A, Kinne D, Mikè V (1977) Medullary carcinoma of the breast. Cancer 40:1365–1385
28. Rigaud C, Theobald S, Noel P, Badreddine J, Barlier C, Delobelle A, Gentile A, Jacquemier J, Maisongrosse V, Peffault de Latour M, Trojani M, Zafrani B (1993) Medullary carcinoma of the breast. A multicenter study of its diagnostic consistency. Arch Pathol Lab Med 117:1005–1008
29. Shek TW, Luk IS, Ng IO, Lo CY (1996) Lymphoepithelioma-like carcinoma of the thyroid gland: lack of evidence of association with Epstein-Barr virus. Hum Pathol 27:851–853
30. Swanson SA, Cooper PH, Mills SE, Wick MR (1988) Lymphoepithelioma-like carcinoma of the skin. Mod Pathol 1:359–365
31. Tseng CJ, Pao CC, Tseng LH, Chang CT, Lai CH, Soong YK, Hsueh S, Jyu-Jen H (1997) Lymphoepithelioma-like carcinoma of the uterine cervix: association with Epstein-Barr virus and human papillomavirus. Cancer 80:91–97
32. Wargotz ES, Silverberg SG (1988) Medullary carcinoma of the breast: a clinicopathologic study with appraisal of current diagnostic criteria. Hum Pathol 19:1340–1346
33. Wu TC, Kuo TT (1993) Study of Epstein-Barr virus early RNA 1(EBER1) expression by in situ hybridization in thymic epithelial tumors of Chinese patients in Taiwan. Hum Pathol 24:235–238
34. Yuen ST, Chung LP, Leung SY, Luk IS, Chan SY, Ho J (1994) In-situ detection of Epstein-Barr virus in gastric and colorectal adenocarcinomas. Am J Surg Pathol 18:1158–1163