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Cytokeratin and Vimentin Expression in Normal Epithelium and Benign Lesions of the Vocal Cords

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The expression of cytokeratins and vimentin was studied immunohistochemically in normal epithelium and 12 benign lesions of the vocal cord with the use of a broad panel of monoclonal antibodies against cytokeratins and vimentin. Histology showed that the various lesions contained hyperkeratotic, hyperplastic and atrophic epithelium, irrespective of their clinical appearance. Especially the Ck profile of the (hyper)keratotic lesions was changed in comparison with the native epithelium. Increased expression of the keratinization marker Ck 10 was associated with decreased expression of the stratification markers Cks 4 and 13. Expression of the basal cell marker Ck 14 and hyperproliferation-associated Cks 16 and 17 was increased in all the benign lesions, except in atrophic epithelium. These expression patterns differ from those observed in malignant epithelial lesions. The latter show a marked expression of simple cell Cks and vimentin and more pronounced expression of hyperproliferation-associated markers than the benign lesions. *Key words: immunohistochemistry, squamous cell carcinoma, larynx, subepithelial blistering.*

INTRODUCTION

Epithelial lesions of the larynx can be benign or malignant. Most of the benign lesions are pseudotumours, such as vocal cord nodules, hyperkeratotic lesions and polyps (1). It is often difficult to make a clinical diagnosis and to distinguish between benign, premalignant and malignant epithelial lesions of the vocal cords (2).

In the past decade, the expression of intermediate filament proteins (IFPs) has been used to further characterize various types of malignancy. IFPs are a major component of the cytoskeleton and are expressed in a tissue-specific fashion. Various cytokeratins (Cks) are specific for epithelial cells and have proven to be important markers for the study of normal and abnormal epithelial differentiation (3). In man these Cks comprise a family of 20 different subtypes which are expressed in relation to the type of epithelial differentiation. In addition, the expression of vimentin, the mesenchymal type of IFP, was studied, because several investigators have established that it is existent in epithelial cells and carcinomas (4).

Ck expression may change during hyperplasia, hyperkeratosis and malignant transformation (5). For example, in squamous cell carcinomas (SCC) of the head and neck, shifts have been observed in the Ck expression patterns as compared with the normal epithelium and amongst the different tumour grades (6). Although several studies have dealt with Ck expression in benign epithelial lesions of the oral

cavity (7–9), to our knowledge, only one study has been published focusing on the larynx (10). These authors investigated the Ck expression in vocal cord polyps using broadly cross-reacting antibodies and reported significant heterogeneity in the Ck subtypes present in the different polyps. In the present study, IFP expression was investigated in normal epithelium and 12 histopathologically different benign epithelial lesions of the vocal cords using a large panel of monospecific antibodies directed against the representatives of the various Ck subsets, as well as a vimentin antibody.

MATERIAL AND METHODS

Tissues

Normal vocal cord epithelium was obtained within 10 h after death from 7 autopsy cases without any history of laryngeal disease. Fresh tissue samples were obtained from clinically benign lesions during microlaryngeal surgery. Immediately after dissection, the specimens were frozen in liquid nitrogen and 5 µm thick cryosections were made. To establish the histological diagnosis the epithelial lesions were evaluated according to the classification of Fitz-Hugh et al. (11) and Loire et al. (12) by two different observers. In the case of discrepancy, consensus was reached by joint re-evaluation. After it had been established histologically that the 12 lesions were benign, additional sections were processed for immunohistochemistry.

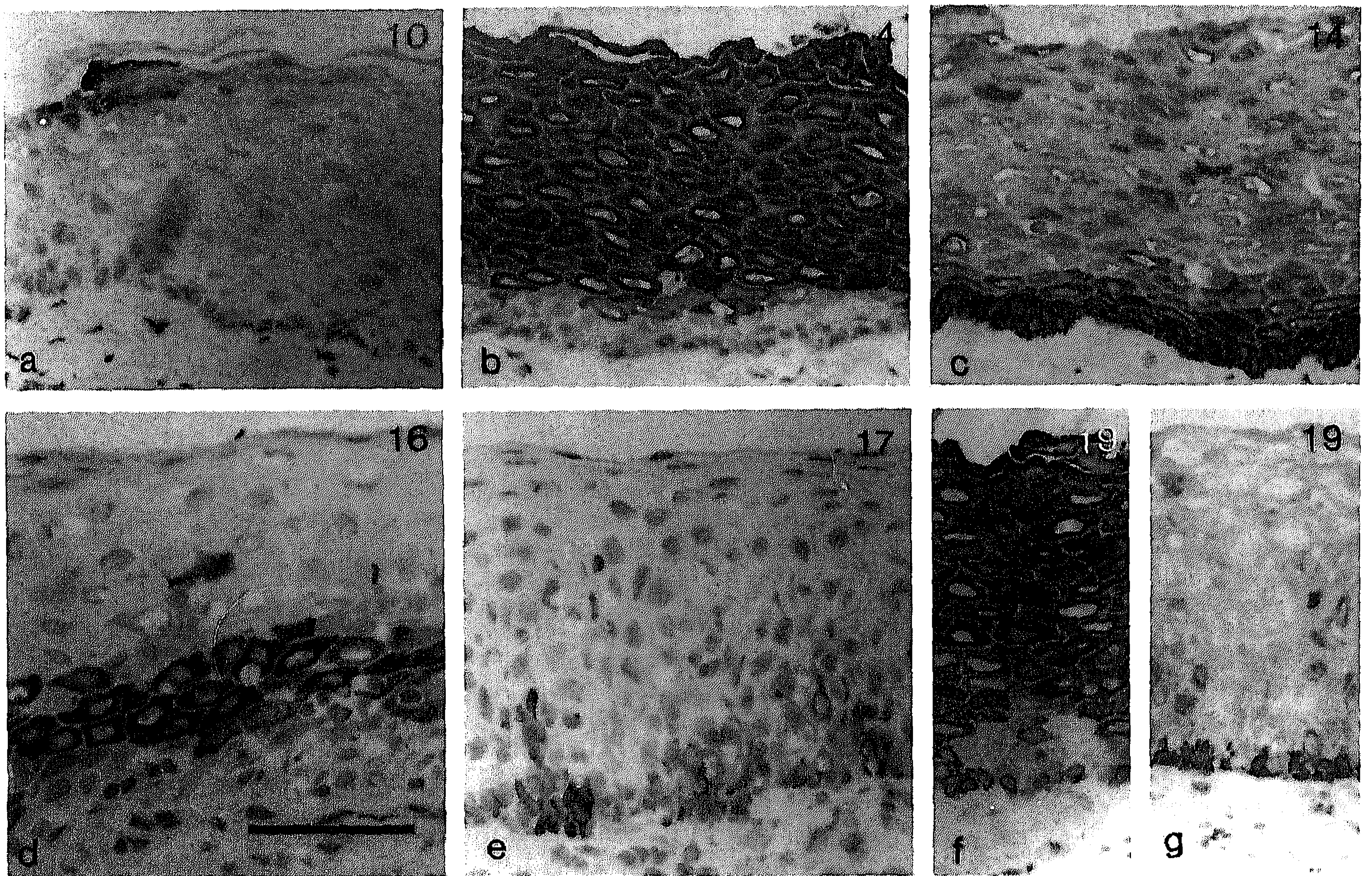


Fig. 1. Ck expression in non-keratinizing squamous epithelium of the normal vocal cords. The arabic numerals refer to the different Ck subtypes. Bar indicates 100 μ m. Note the different expression patterns of Ck 19 in the NKSE adjacent to immature stratified epithelium (f) and in the NKSE (g).

Immunohistochemistry

Immunohistochemistry was performed according to the indirect immunoperoxidase technique as reported previously (13). The staining patterns were recorded semi-quantitatively. Positive staining of 75% of the target cells was defined as homogeneous staining pattern. Positive staining of 25–75% of the target cells was defined as heterogeneous staining. Staining of fewer target cells was defined as sparse or scattered staining pattern.

Antibodies

Twenty-two different mouse monoclonal antibodies (MAbs) directed against individual Cks and one monoclonal antibody to vimentin were applied in this study. The following monoclonal antibodies were used: RKSE60 (Ck 10), 6B10 (Ck 4), 2D7 and 1C3 (Ck 13), AE14 (Ck 5), RCK107, LL001 and LL002 (Ck 14), KA12 (Ck 6), LL025 (Ck 16), E3 (Ck 17), RCK105 and OVTL12/30 (Ck 7), CAM 5.2 and M20 (Ck 8), 2C8, RGE53, CK18.2 and RCK106 (Ck 18), LP2K and RCK108 (Ck 19) and RV202 (vimentin). For source and further specifications of these antibodies see reference (14).

RESULTS

Histology

In all the specimens, the normal epithelium of the vocal cords of the autopsy cases consisted of non-keratinizing squamous epithelium (NKSE), while the ventricle was lined with immature squamous epithelium and pseudo-stratified epithelium. The subglottic region was also lined with pseudo-stratified epithelium.

The 12 benign lesions comprised four nodules, three polyps, one squamous papilloma, two lesions with hyperkeratosis and two lesions which showed non-specific chronic inflammation. The histological features of the lesions varied widely between the different specimens and also within the same specimen. They comprised (hyper)keratinization, atrophy and hyperplasia, while atypia was observed incidentally. Epithelial hyperplasia was mainly observed in the laryngeal polyps and papillomatosis and in the two lesions with non-specific inflammation. Atrophic epithelium was observed in the laryngeal nodules. The hyperkeratotic lesions mainly showed parakeratosis and orthokeratosis, while the squamous papil-

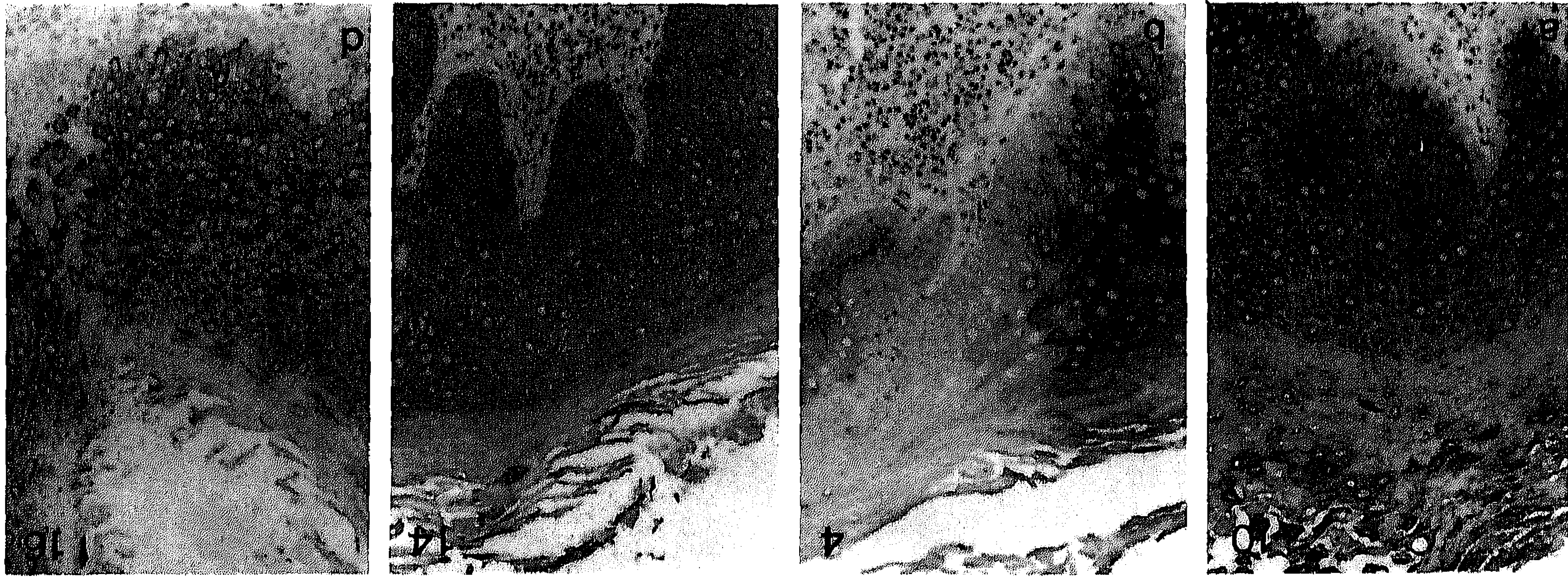


Fig. 2. Ck expression patterns in hyperkeratotic lesions of the vocal cord. The arabic numerals refer to the different Ck subtypes. Bar indicates 100 μ m.

sparse (Fig. 3A). In the lesions with laminares desquamation and in atrophic epithelium, Ck 10 expression was only apparent when there was (pre)keratinization (Fig. 4A).

Stratification markers Cks 4 and 13: In the normal epithelium, Cks 4 and 13 showed homogeneous expression in the suprabasal cell layers and a scattered expression in the basal and parabasal cell layers (Fig. 1B).

In all the lesions, Cks 4 and 13 were expressed suprabasally, but this pattern differed in relation to the epithelial phenotype. In the hyperkeratotic lesions, the expression of Cks 4 and 13 was sparse to heterogeneous (Fig. 2B). In areas of hyperplasia, the stratification markers were heterogeneously expressed in the suprabasal cell layers (Fig. 3B). The atrophic epithelium showed homogeneous suprabasal expression, with exception of the parabasal cell layer (Fig. 4B). In the areas of blistering expression of Cks 4 and 13 was greatly decreased (Fig. 5A). *Basal cell markers Cks 5 and 14:* In the normal epithelium, Cks 5 and 14 were expressed homo-

lyoma, the laryngeal polyps and the nodules showed sparse cornification.

A localized distention of the tissue between the lamina propria and the epithelium referred to as "blistering" was observed in 3 of the lesions (one polyp, one hyperkeratotic lesion and one lesion with non-specific inflammation), while 2 of them (the polyp and the lesion with non-specific inflammation) showed extensive subepithelial accumulation of lymphocytes.

Intermediate filament protein expression

The Ck profile is described according to their reactivity pattern of the different types of epithelial differentiation present in the various specimens. The results are depicted in Figs. 1–5.

Cornification marker Ck 10: In epithelium of the vocal cord, only scattered suprabasal cells reacted with the Ck 10 antibody (Fig. 1A). In the lesions with keratinization there was an abundant expression of Ck 10 in the suprabasal cell layers (Fig. 2A). The expression of Ck 10 in hyperplastic epithelium was

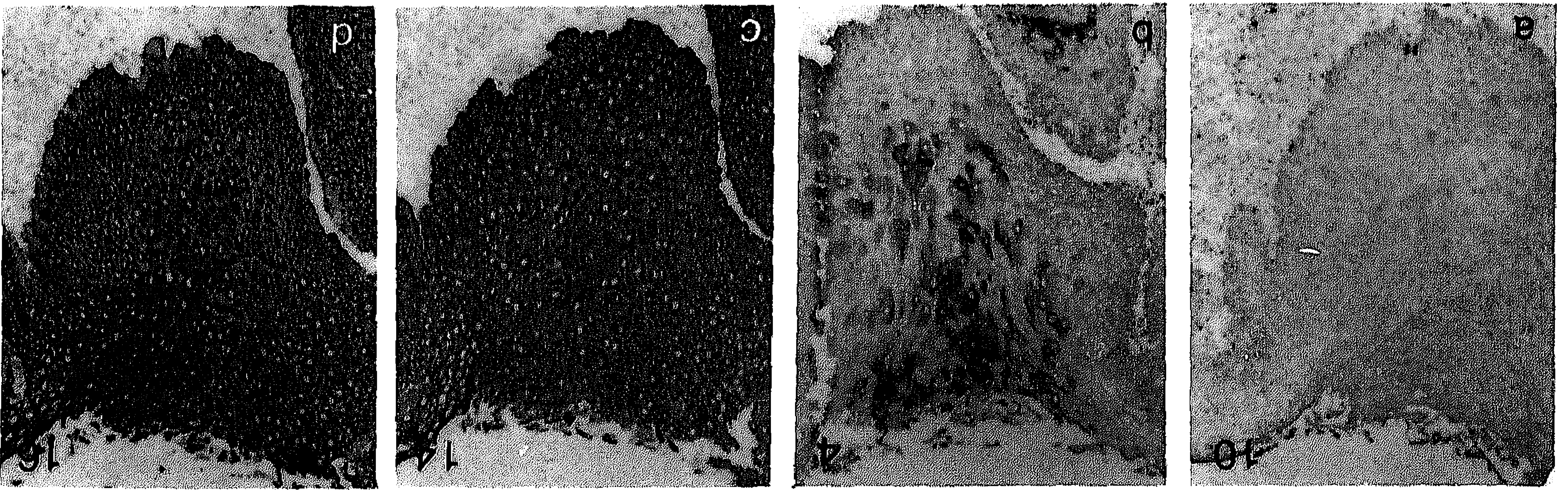


Fig. 3. CK expression patterns in hyperplastic epithelium of benign lesions of the vocal cord. The arabic numerals refer to the different CK subtypes. Note the expression of CK 7 in the uppermost cell layer of a lesion with hyperplastic epithelium, lying adjacent to immature squamous epithelium (g). Bar indicates 100 μ m.

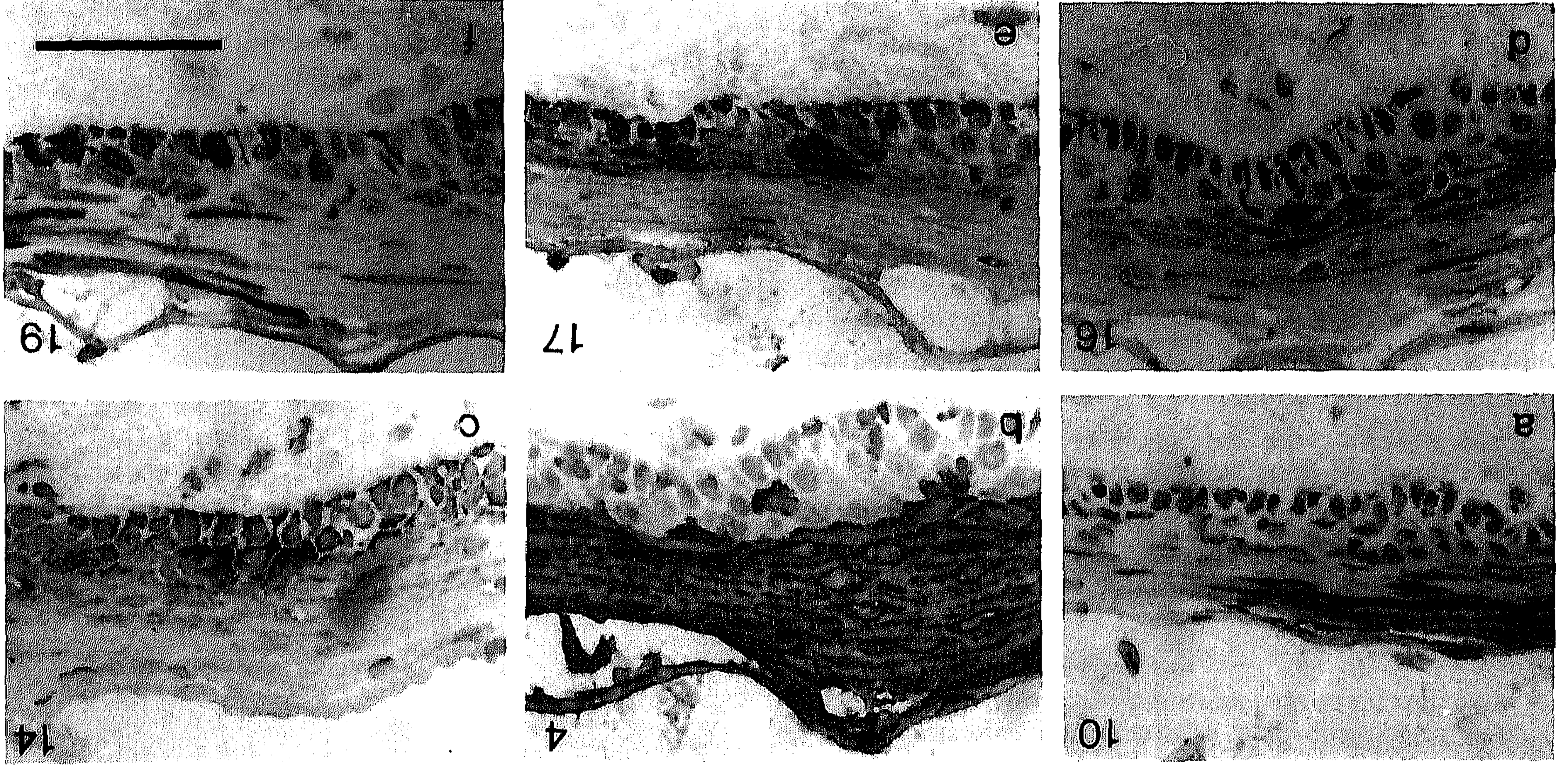


Fig. 4. CK expression patterns in atrophic epithelium of benign lesions of the vocal cord. The arabic numerals refer to the different CK subtypes. Bar indicates 50 μ m.

neously in the basal and parabasal cell layers (Fig. 1C). In the basal and parabasal cell layers of the benign lesions the expression of CK 5 was generally sparse. Irrespective of their type of differentiation, most of the lesions showed homogeneous expression of CK 14 in all the cell layers (Figs. 2C and 3C). In the atrophic epithelium, CK 14 expression was im-

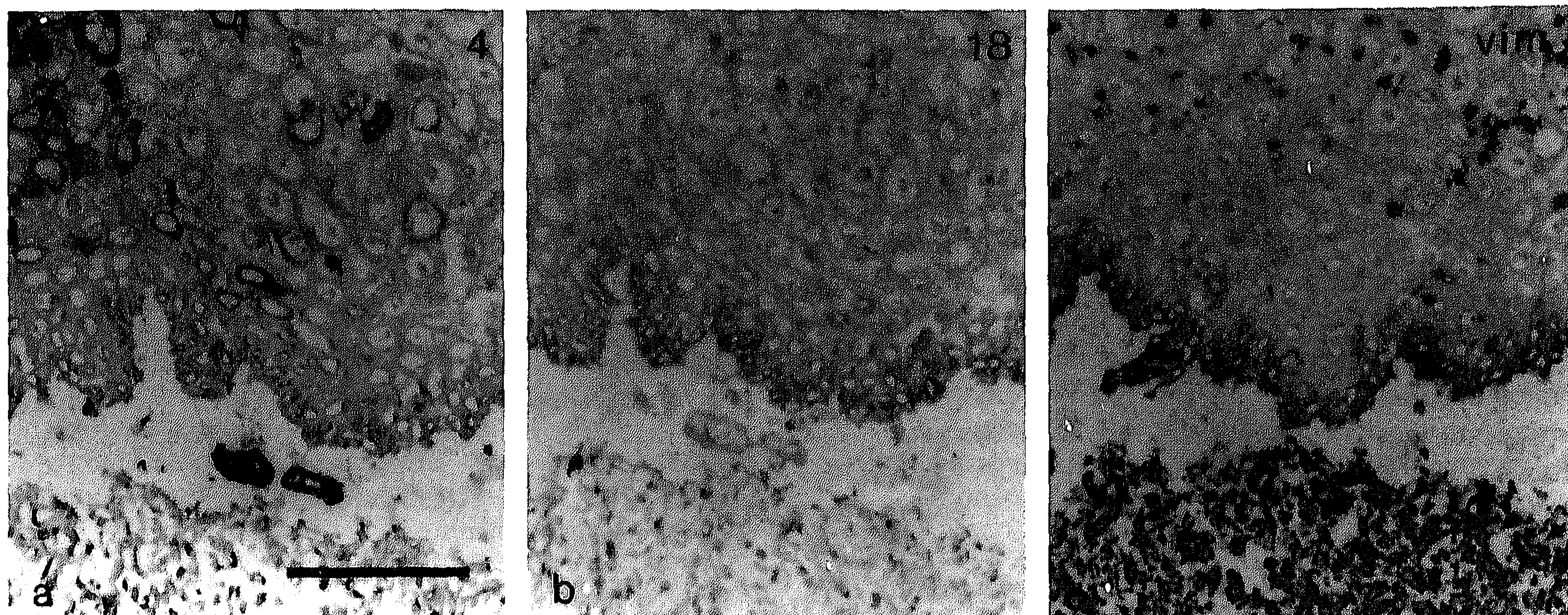


Fig. 5. Expression of Ck 4 (A), Ck 18 (B) and vimentin (vim) (C) in an area of blistering in a lesion with hyperplastic epithelium. Expression of Ck 4 is decreased in the area above the blister, while Ck 18 and vimentin are exclusively present in the basal cells lining the blister. Bar indicates 100 μ m.

ited to the basal and parabasal cell layers (Fig. 4C).

Hyperproliferation markers Ck 6, 16 and 17: The normal epithelium of the vocal cords showed homogeneous expression of Ck 6 in the suprabasal cell layers, whereas the basal cell layer showed a heterogeneous expression. Ck 16 was expressed in scattered areas of suprabasal cells (Fig. 1D), while Ck 17 showed heterogeneous expression in the (para)basal cells (Fig. 1E).

In all the lesions Ck 6 was expressed homogeneously. Keratotic lesions and hyperplasia showed heterogeneous expression of Cks 16 and 17 in all the suprabasal cell layers (Figs. 2E and 3E), while Ck 16 was also expressed heterogeneously in the basal cell layer of both lesions. In atrophic lesions, Ck 16 expression was limited to the suprabasal cell layers in areas with keratinization (Fig. 4D), while Ck 17 only was expressed in the basal cell layer (Fig. 4E).

Simple epithelium-related markers Cks 7, 8, 18 and 19: Cks 7, 8 and 18 were not expressed in the normal epithelium of the vocal cords. Ck 19 was expressed heterogeneously in the basal cells and focally in the suprabasal cells. The epithelium of the vocal cord adjacent to the ventricle and subglottic region expressed Cks 7, 8 and 18 in the uppermost cell layers, while Ck 19 was expressed heterogeneously in all cell layers in these areas (Fig. 1F). This expression profile was also found in the epithelial lesions bordering the ventricle and subglottic region (Fig. 3G).

Generally, Cks 7, 8 and 18 were not expressed in the lesions, except in the lesions with subepithelial blistering, which showed heterogeneous expression of Cks 8 and 18 in the basal cell layers (Fig. 5B).

Expression of Ck 19 was limited to the hyperplastic and atrophic epithelium. The hyperplastic epithelium showed heterogeneous expression of Ck 19 in all the cell layers, but expression could differ widely at the various sites (Fig. 3F). In atrophic epithelium, Ck 19 was expressed heterogeneously in the basal cells and scattered in the suprabasal cell layers (Fig. 4F). In the hyperkeratotic lesions, there was little or no expression of Ck 19 (Fig. 2F).

Vimentin: Vimentin expression in normal vocal cord epithelium was limited to mesenchymal cells of the connective tissue. This also applied to the lesions. The lesions with subepithelial blistering showed a distinct expression of vimentin in the basal cells bordering the blister (Fig. 5C).

DISCUSSION

The present study demonstrates that clinically distinguishable benign lesions of the vocal cord can show a variety of epithelial phenotypes. These lesions were characterized by (hyper)keratosis, hyperplasia and atrophy. In addition, the lamina propria also showed various changes.

Our immunohistochemical data on the presence of IFPs showed that the original expression pattern of various Cks had changed. In particular, these changes were evident in the keratinizing and hyperplastic epithelium. They were characterized by increased expression of the keratinization marker Ck 10, the basal cell marker Ck 14 and hyperproliferation-associated Cks 16 and 17. The Ck expression pattern of atrophic epithelium largely resembled that of normal epithelium, except in the keratinized areas.

The expression of Ck 10 was clearly related to keratinization and was often associated with a decrease in the stratification markers Cks 4 and 13. This inverse relation is in line with observations made by others in benign epithelial lesions of the oral cavity (15).

Extension of basal cell marker Ck 14 into the suprabasal cell layers and the upregulation of Ck 16 indicates hyperproliferation in the benign vocal cords lesions, as it does in other epithelial abnormalities (16, 17). In contrast with Ck 14, expression of the other basal cell marker Ck 5 did not extend into the suprabasal cells, although it has been described that these two Cks form copolymers (18). This phenomenon is difficult to explain, but a comparable dissociation between the expression of CKs 5 and 14 has been reported in skin under hyperproliferative conditions (19).

In normal NKSE, Ck 17 is expressed in the basal cell layers. In several of the studied lesions the expression of Ck 17 had shifted to the suprabasal cell layers, while expression had decreased in the basal layers. Although Ck 17 is considered to be a marker of basal cells of non-keratinizing epithelia (20), the fact that its expression pattern is similar to that of Ck 16 in epidermal lesions (21) suggests that Ck 17 can be considered as a hyperproliferation-associated cell marker under these conditions.

The simple epithelial cell markers Cks 8 and 18 were not expressed in the benign lesions, except for the basal cells bordering the fluid-filled blisters. This suggests that there may be a relationship with this peculiar anatomical position, i.e. bordering a fluid-filled cavity. The expression of vimentin in these cells can indicate that they have special properties. The latter observation agrees with the suggestion made by Kasper et al. (22) that vimentin is often expressed in the epithelial lining of fluid-filled cavities.

Ck 19 is generally considered to be a marker of simple and complex epithelia, but it is also present in the basal cells of non-keratinizing epithelia in areas of variable or labile differentiation, where two types of differentiation coexist, such as the mouth and cervix (23). Its extension into the suprabasal cell layers can be assumed to indicate disturbance of differentiation.

The expression patterns of Cks 5, 14, 4 and 13 in benign lesions were similar to those in larynx carcinoma, but the expression of hyperproliferation-associated Cks 16 and 17 was more pronounced in carcinoma (van der Velden et al. unpublished observations). In addition, keratinocytes in carcinoma show marked expression of simple cell markers Cks 8 and 18, and often of vimentin. The same applies to Ck 19, although some carcinomas can be completely devoid of Ck 19. According to Lindberg et al. (24) and Copper et al. (25) the expression of Ck 19 in

normal NKSE and benign lesions of the oral cavity is related to premalignancy. However, the present observations of Ck 19 expression in normal NKSE and in benign laryngeal lesions does not support this assumption.

We conclude that benign lesions of the vocal cords differ from squamous cell carcinomas of the larynx in the expression pattern of simple cell Cks and vimentin. This may be of use in case of differential diagnosis.

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