Morphopathological stigmata in acne fulminans

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Abstract

Acne fulminans is the most aggressive and destructive form of acne vulgaris, being also known as acne maligna. The onset is acute and systemic involvement is always present. Most commonly, acne fulminans (AF) occurs in male adolescents as a brutal complication of a preexisting mild or moderate acne. The etiology of AF remains incompletely elucidated. The skin lesions are polymorphic, the symptoms and clinical signs vary, and thus the diagnosis is not easy. In making a certain diagnosis of AF, histopathology has a decisive role. In this respect, we will present some of the most suggestive aspects of histopathology, immunohistochemistry and electron microscopy in a 16-year-old patient clinically diagnosed with AF. This patient presented on admission nodular inflammatory and ulcerative necrotic lesions on the face and chest, extremely, accompanied by significant myalgias and arthralgias.

Keywords: acne fulminans, nodular and ulcerative necrotic lesions, histopathology, immunohistochemistry.

Introduction

Acne fulminans (AF), also known as acne maligna is a rare and severe disease, a form of nodular and ulcerative necrotic acne vulgaris. The onset is acute and systemic involvement is always present, the severity of symptoms and signs varying from case to case.

AF was first described in literature in 1959 by Burns & Colville, who considered it “acne conglobata with septicemia”. In 1971, Kelly & Burns introduced the term “acute febrile ulcerative acne conglobata with polyarthralgias”. The term AF was proposed in 1975 by Plewig & Kligman [1]. Later, Goldschmidt et al. [2] defined AF as a rare disorder of male teenagers characterized by sudden onset of highly inflammatory and ulcerative necrotic lesions on the dorsal chest, pre sternal, shoulders, arms and more rarely on the face. Lesions always occur in patients with mild to moderate acne. The general health status is impaired, with fever, loss of appetite, weight loss, myalgias, arthralgias, polyarthritis of large joints [3]. Sometimes, erythema nodosum, aseptic osteolysis, hepatosplenomegaly or myositis may be also associated [4, 5]. In the literature, there are case reports of AF associated with pyoderma gangrenosum or SAPHO (synovitis, acne, pustulosis, hyperostosis, osteitis) syndrome [6]. AF affects 1% of the population with acne vulgaris, being the most severe form [7]. The incidence of AF is decreasing, most likely due to early and adequate treatment of mild or moderate acne.

The etiology of AF remains unknown. The infectious factor is excluded due to the negative hemocultures and bacterial cultures of lytic bone lesions, as well as to the antibiotic resistance of this form of acne. Drug etiology still remains debatable, some literature data supporting the efficacy of isotretinoin, others reporting the onset of AF during isotretinoin therapy. The most plausible cause remains the increased hypersensitivity reactions to Propionibacterium acnes antigens, supported by the existence of circulating immune complexes (CIC) to P. acnes and the favorable response to systemic corticosteroids [8].

Case report

A 16-year-old patient presented on admission, to the Iassy Dermatology Clinic, extensive, very painful inflammatory nodulocystic skin lesions and ulcerative necrotic lesions (Figure 1) located on the dorsal thorax, shoulders and arms (Figure 2), pre sternal and mandibular region. The lesions tended to extend and group in ulcer craters. Ulcer base is an amorphous, gelatinous, necrotic mass. On the face and forehead were present only comedones, papules, pustules and rare nodules.

Patient’s general health status was altered, with fever (38.5°C), loss of appetite, weight loss, myalgias, arthralgias, anxiety and swelling of the right knee.

Laboratory tests

The performed laboratory tests demonstrated the presence of a biological inflammatory syndrome (elevated ESR – erythrocyte sedimentation rate, leukocytosis, in-
creased fibrinogen, increased C-reactive protein), increased serum complement C3 level, increased IgA and the presence of CIC. Bacteriological examination of pus from a pimple revealed the presence of *P. acnes* associated with *Staphylococcus epidermidis*.

Rheumatology consultation made a diagnosis of reactive arthritis in the right knee but without radiological changes.

In making a certain diagnosis of AF, histopathological investigation is welcome. In current practice, the only investigation used is histopathology, while immunohistochemistry and electron microscopy are used rarely, mostly for research purposes.

Skin biopsies were prepared according to the routine histological technique at the Department of Histology, “Grigore T. Popa” University of Medicine and Pharmacy and Laboratory of Pathology, “Sf. Spiridon” Emergency Hospital, Iassy, Romania.

The obtained fragments were fixed in 15% formalin, embedded in paraffin, and the 7–10 μm thick sections were stained with Hematoxylin–Eosin (HE), trichrome Szekely, Periodic Acid–Schiff (PAS) and Orcein.

Histopathological appearance of AF was impressive. It revealed an ulceration, which included epidermis and dermis, with necrotic-leukocytary detritus in the base, papillomatous thickening of the epidermis in the slope, with discrete hyperkeratosis. Perivascularly, in the papillary and middle dermis it was noticed the presence of a fibrino-necrotic exudate and a polymorphous inflammatory infiltrate with integer and lysed PMNs (polymorphonuclears).

In AF, comedones were present in small numbers and with an intense inflammatory reaction in the surrounding skin (Figure 3). Pilosebaceous follicles in the affected areas were surrounded by an intense foreign body granulomatous reaction. The inflammatory reaction extended to the middle and deep dermis and was polymorphic, consisting of PMNs, macrophages, lymphocytes and plasma cells.

Elastic fibers of the dermis were fragmented, thickened, chaotically arranged or even absent in the area of inflammation and necrosis (Figure 4).

The study was conducted in collaboration with the Laboratory of Electron Microscopy at the Department of Cellular and Molecular Biology, “Grigore T. Popa” University of Medicine and Pharmacy, Iassy. The purpose of the electron microscopy was to identify the ultrastructural changes specific to this pathology.

The samples collected from the acne lesions had been processed in accordance with the transmission electron microscopy techniques.

In AF, the inflammatory infiltrate in the papillary and middle dermis consisted of lymphocytes and macrophages. Activated lymphocytes and macrophages showed cytoplasmic vacuolation and presence of *P. acnes* in the cytoplasm of macrophages. Because of *P. acnes* phagocytation, the ultrastructure of macrophage was modified, presenting numerous lysosomal vacuoles in which partially altered *P. acnes* was found.

Because of the chronic inflammatory process, numerous fibroblasts became activated, producing a large amount of collagen fibers, arranged in strips different directions. Capillary vessels in the inflammatory infiltrate had unequal, stenosed lumen due to hypertrophy of the endothelial cells protruding into the lumen, and the basement membrane is uniformly thickened.

Immunohistochemical study was conducted in collaboration with the Laboratory of Immunology, “Sf. Spiridon” Emergency Hospital and aimed at investigating the inflammatory reactions associated with typical acne lesions and identification of the cell types involved in triggering and modulating these reactions. Another aim was the assessment of angiogenesis, an important element in the progression of chronic inflammation, and keratinization disturbances known to occur in the pilosebaceous follicle in the infrainfundibular region. Also followed were the possible immunohistochemical changes that may be markers of sebaceous gland hyperplasia and hypersecretion in areas with acne lesions.

To this end, we investigated the expression of some specific markers for T-lymphocytes (CD3 antigen), B-lymphocytes (CD20 molecule), monocyte/macrophage lineage (CD68 molecule), epithelial cells (cytokeratins) and endothelial cells (CD34 II molecule) (Table 1).

<table>
<thead>
<tr>
<th>No.</th>
<th>Primary antibody</th>
<th>Antigenic substrate (ligand)</th>
<th>Source</th>
<th>Dilution / Incubation time</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Cytokeratin</td>
<td>Keratinocytes 5, 6, 8, 17 and 19 (with low and medium molecular weight 45–56.5 kDa)</td>
<td>Clone MNF116 (mouse anti-human), Dako, Denmark</td>
<td>1:100 / overnight, 4°C</td>
</tr>
<tr>
<td>2.</td>
<td>CD34 II</td>
<td>Endothelial cells</td>
<td>Clone QBEnd10 (mouse anti-human), Dako, Denmark</td>
<td>1:50 / overnight, 4°C</td>
</tr>
<tr>
<td>3.</td>
<td>CD20</td>
<td>B-lymphocytes</td>
<td>Clone L26 (mouse anti-human), Dako, Denmark</td>
<td>Ready-to-use / 10 minutes, room temperature (RT)</td>
</tr>
<tr>
<td>4.</td>
<td>CD3</td>
<td>T-lymphocytes</td>
<td>Polyclonal (rabbit anti-human), Dako, Denmark</td>
<td>Ready-to-use / 30 minutes, RT</td>
</tr>
<tr>
<td>5.</td>
<td>CD68</td>
<td>Macrophages</td>
<td>Clone PG–M1 (mouse anti-human), Dako, Denmark</td>
<td>Ready-to-use / 10 minutes, RT</td>
</tr>
</tbody>
</table>
The employed method was indirect immunohistochemical staining that involves an unlabeled primary antibody specific to the target antigen. An enzymatically labeled secondary antibody reacted with the primary antibody, and the reaction was visualized with a chromogenic substrate.

The study was performed on paraffin-embedded sections from skin biopsies collected from patients with acne vulgaris, the patient diagnosed with AF included.

After washing with distilled water, the sections were counterstained with Hematoxylin for two minutes at room temperature, followed by washing with distilled water, 100% ethanol and benzene/xylene.

Sections were investigated with a Nikon microscope and the presence of specific markers was highlighted by the appearance of a brown precipitate in the membrane (CD3 and CD20) or cytoplasm (cytokeratins, CD68, CD34 II) corresponding to the identified antigen. Specific marking of the epithelial cells by anti-cytokeratin antibody was evaluated qualitatively positive versus negative, for different types of lesions or epithelial structures present in the skin biopsies. We established three degrees of cytokeratin immunostaining intensity: + (weak intensity), ++ (medium intensity), and +++ (high intensity).

The inflammatory infiltrate was assessed for each staining by counting the marked cells and expressing the results as a percentage of total cells present in a microscopic field.

Other sections were subjected to the same protocol, except for the primary antibody incubation step, which were replaced with antibodies of the same isotype, but with irrelevant specificity, serving as negative controls.

In the studied acne cases, AF included, cytokeratin staining was present in the epidermal keratinocytes, cells of pilosebaceous follicle, sebaceous and apocrine sweat glands (Figure 5).

Staining intensity was high (+++) in the supporting cells of the hypertrophied sebaceous acini (Figure 6), which would suggest a high-level expression. In the pilosebaceous follicle, in the infrainfundibular area, dyskeratotic, vacuolated keratinocytes appear to be less stained (+) (Figure 7). Comedones, also present in AF, showed staining for cytokeratins only in the follicular wall (Figure 8). Macrophages in the perifollicular dermis had specific receptors able of being activated by *P. acnes*, amplifying the inflammatory reaction.

In AF, the dermal perifollicular inflammatory infiltrate was dominated by CD3+ cells (T-lymphocytes) and CD68+ cells (of the mononuclear phagocyte system) (Figure 9), fact supporting the hypothesis of the involvement of delayed hypersensitivity reaction (type IV) in the pathogenesis of acne. CD3+ cells accounted for 40–50% of all cells in the perifollicular inflammatory infiltrates.

CD68+ macrophages were present in all cases of acne studied, regardless of the type of inflammatory lesion. Their number was higher in AF, being located in the close vicinity of dilated and broken follicle wall, and some of them presenting as epithelioid cells and giant cells, additional argument supporting the cell-mediated hypersensitivity as a major pathogenic mechanism in acne. Together with lymphocytes and neutrophils, they might constitute the foreign body granulomatous reaction.

CD20+ cells (B-lymphocytes) (Figure 10) were found in small numbers and only in the severe forms of acne, including AF (less than 20 cells in the entire inflammatory infiltrate).

Chronic inflammatory inflammation, characteristic to acne lesions, was also accompanied by angiogenesis. By specific immunostaining for CD34+ endothelial cells, our study revealed the presence of blood capillaries around the pilosebaceous follicles, within the inflammatory or pericystic infiltrate (Figure 11).
**Discussion**

Acne fulminans is a very rare, severe manifestation that can develop during the course of acne vulgaris [5]. It develops mostly on male adolescents, aged 13 to 16 years, with mild to moderate acne [5, 7, 9].

Karvonen [10] set absolute (severe ulcerated nodulo-cystic acne with acute onset; exercise arthralgias and/or myalgias stress – at least one week) and relative criteria (fever over 38°C, at least one week; leukocytosis over 10 000/mm³; ESR over 50 mm/hour; C-reactive protein over 50 mg%; and osteolytic lesions on X-rays, bone scintigraphy or tomography) for positive, clinical and laboratory diagnosis of AF. The diagnosis of AF is made in the presence of two absolute criteria and two relative criteria.

The reported case represents a typical case of AF, accomplishing the diagnostic criteria. Thus, the patient presented extensive, very painful inflammatory nodulo-cystic and ulcerative skin lesions located on the dorsal thorax, shoulders, arms, presternal and mandibular region, associating myalgias, arthralgias and swelling of the right knee, fever. The laboratory test demonstrated the presence of biological inflammatory syndrome.

The diagnosis of AF is made in the presence of two absolute criteria and two relative criteria.

Histopathological changes underlying the AF clinical lesions have been described in numerous studies and treatises of both dermatology and histopathology [11–13].

Kligman [11] wrote a monograph that included a description and images of the different forms of acne vulgaris, AF included, which we found on the histological
sections from skin biopsy performed in our AF patient. Weedon [12] also included in their book the acne fulminans’ histopathological description, which was in concordance with our results.

In AF, the skin shows large areas of necrosis, with ulcerations covered by parakeratotic crusts formed by agglomeration of PMNs. Ulcer base is occupied by a necrotic and fibrinoid dermis and edema. Inflammatory infiltrate contains intact and lysed PMNs and incorporates partially destroyed vessels and apocrine sweat glands [11]. As well as in the literature, to our patient, the inflammatory reaction extended to the middle and deep dermis, consisting of PMNs, macrophages, lymphocytes and plasma cell [8]. Also, sometimes microabscesses form and dissect the dermis in different direction or sinuous tracts [14].

The results of few electron microscopic studies on AF cases are published in the literature [10, 11, 15].

In AF, *P. acnes* could be also detected in perifollicular dermal macrophages, but at extracellular, in their vicinity. Also, besides the intense destructive processes of the epidermis and dermis intense as a result of an extensive inflammatory process evolving toward necrosis large areas of fibrosis do occur [16]. As well as in the literature [17], we also found numerous lysosomal vacuoles in which partially altered *P. acnes* was found, and a large collagen fibers, arranged in strips different directions, consequence of the chronic inflammatory process.

Immunohistochemical investigations of acne lesions, AI included, are extremely few worldwide.

Cytokeratins or prekeratins are part of a system of intermediate filaments, which is found in the cytoplasm of simple and multilayered epithelia, keratinized or non-keratinized.

Intermediate filaments are a part of the cytoskeleton of all cellular elements and, depending on their protein structure, are divided into five classes: cytokeratins, vimentin, desmin, neurofilaments, and glial filaments.

Moll divided cytokeratins into three groups and has numbered them 1 to 20, as follows: high molecular weight (MW) (1–2, 3, 9, 10–11), medium MW cytokeratins (4, 5, 6, 13, 14–15, 16) and the low MW cytokeratins (7, 8, 17, 18, 19, 20) [18, 19].

Expression of cytokeratins in normal skin varies depending on the position of keratinocytes in the various layers of the epidermis. Thus, basal layer cells express cytokeratins 5, 14–15, 17 and 19 (according to Moll classification) [19]. The cells of spinous, granular and shiny layers are positive for cytokeratins 4, 5, 10–11, 13, 14–15, 17 and 19. Stratum corneum presents cytokeratins 1–2, 6, 10–11 and 16. Corneocytes that desquamate are marked by cytokeratins 1–2, 9, 10–11 [20].

There are studies confirming the marked proliferation of keratinocytes in the infrainfundibular portion of the pilosebaceous follicle by increasing the expression of cytokeratins 6 and 16, at their level, in acne patients or in those likely to develop acne compared to normal individuals. Another study, which used the monoclonal antibodies Ki-67 that fixes on nuclei of cells undergoing division, showed their marked expression in the keratinocytes of comedone wall compared to the normal pilosebaceous follicle wall.

Sweat gland secretory cells are positive for cytokeratins 7, 18, 19, and myoepithelial cells have immunoreactivity for cytokeratins 5, 14 and 15. Excretory duct cells are positive for cytokeratins 8, 14, 17, 19.

Sebocytes have as markers medium of high MW cytokeratins.

The used anti-Cyt antibody (Dako, Denmark) has specificity for cytokeratins 5, 6, 8, 17 and 19 in the basal, spinous and granular layers of the epidermis and pilosebaceous follicles, as well as at the level of sweat and sebaceous glands.

Stratum corneum and desquamated corneocytes do not express these cytokeratin, and therefore are negative for this type of immunomarking.

The intense positive reaction to Cyt of sebocytes from hypertrophied glandular acini, present in acne lesions, demonstrates an intense proliferation and differentiation of these cells, as well as increased sebum synthesis. All these changes confirm that hyperseborrhea is an etiopathogenic factor of the disease.

Acroinfundibular keratinocytes have a weaker reaction to Cyt staining, explained by the fact that they are dyskeratotic, vacuolated, filled with lipid inclusions.

CD20 is a membrane antigen that marks B-lymphocytes. The inflammatory infiltrate contains few CD20+ cells only in severe forms of acne.

Anti-CD3 antibody reacts with CD3 antigen at the level of T cells. CD3+ cells were found in all acne cases, highlighting the intervention of cellular immunity in triggering the inflammatory reaction in acne, by a type IV hypersensitivity process [21].

CD68 is a 110-kDa glycoprotein, associated to lysosomes, marker for cells of the monocyte/macrophage lineage. In acne lesions there are numerous CD68+ cells, representing macrophages, epithelioid cells and giant cells, which together with T-lymphocytes and PMNs give rise to granulomatous reactions, similar to those of foreign body granuloma [22].

This granulomatous reaction can be explained by the behavior of the content of the comedone (including sebum) as a “non-self” antigenic complex against which the immune system triggers a delayed-type hypersensitivity reaction (type IV).

CD34 II is a monoclonal antibody recognizing an antigen of vascular endothelium. Proliferating endothelial cells with intense activity express this molecule in higher amount than resting cells. In acne lesions, there is a large number of perifollicular dermal capillaries and within the inflammatory infiltrate, but also an intense immunomarking of CD34 II+ endothelial cells [23]. This aspect pleads for the chronicity of the inflammatory reaction associated to acne [24].

**Conclusions**

Acne vulgaris (AV) is a common skin disorder among adolescents, characterized by a chronic but benign, self-limiting course. Acne fulminans (AF), the destructive and aggressive form of AV is an extremely rare skin condition with severe course, which in the absence of specific treatment may be life-threatening. Early clinical diagnosis and laboratory investigations, especially the morphopathological ones, enabled the correct classification of AF
among the severe skin diseases, opening new horizons for research in this field. The etiopathogenesis of AF is not fully known. In literature, there are data on the histopathological changes, but very few data on immunohistochemistry and electron microscopy studies in AF. Therefore, our results raise new hypotheses that deserve further study in larger groups of patients.

Conflict of interests
The authors declare that they have no conflict of interests.

Author contribution
All authors have equally contributed to the realization of the article.

References

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