NASZA DERMATOLOGIA Online OUR DERMATOLOGY Online	ROULEAUX AND AUTOAGGLUTINATION OF ERYTHROCYTES ASSOCIATED WITH FIBRIN-LIKE
	MATERIAL IN SKIN BIOPSIES FORM PATIENTS WITH AUTOIMMUNE BLISTERING DISEASES
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### Abstract

**Introduction:** Autoimmune bullous skin diseases (ABDs) represent a heterogeneous group of disorders of the skin and mucosa; these disorders are commonly associated with deposits of immunoglobulins, complement, and fibrinogen, usually directed against distinct adhesion molecules.

**Methods:** We utilized hematoxylin and eosin (H & E) stained tissues sections to evaluate for the presence of rouleaux in lesional skin biopsies of patients affected by ABDs including patients with endemic and nonendemic pemphigus foliaceus, bullous pemphigoid (BP), pemphigus vulgaris (PV), dermatitis herpetiformis (DH), and a group of controls taken from routine biopsies seen in our practice.

**Results:** Most autoimmune bullous skin diseases biopsies showed rouleaux formation within and around post-capillary venules in the superficial vascular plexus in association with a pinkish brush-like material that resembles fibrin or other amorphous eosinophilic material.

**Discussion:** We document that rouleaux and the pinkish aggregates are present in within biopsies taken from lesional skin in the majority of patients with ABDs and speculate that this maybe as result of the exocytosis of inflammatory cells, antibodies that form when exposed to the extracellular matrix which is already edematous in most ABDs. In addition red blood cells in the presence of plasma proteins or other macromolecules may form aggregates. Further studies are needed.

Key words: Autoimmune blistering skin diseases; rouleax; fibrin; red blood cells

Abbreviations and acronyms: Red blood cells (RBCs), autoimmune bullous diseases (ABDs), bullous pemphigoid (BP), immunohistochemistry (IHC), direct and indirect immunofluorescence (DIF, IIF), hematoxylin and eosin (H & E), pemphigus vulgaris (PV), autoimmune blistering skin diseases (ABD), dermatitis herpetiformis (DH), endemic pemphigus foliaceus (EPF), with linear IgA (LAD).

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# Introduction

Autoimmune bullous diseases (ABDs) are bullous dermatoses of the skin and mucosae characterized by the presence of tissue-bound and circulating antibodies directed against specific target antigens. These diseases comprise two main subgroups, i.e, subepidermal autoimmune bullous disorders and intraepidermal ones such as the pemphigus family [1,2]. It is known that in the majority of ABDs the interstitial fluid volume is increased as determined by the sodium thiocyanate method. Further, this finding can be seen in the upper dermis with hematoxylin and eosin (H & E) [3,4]. We have noticed the presence of rouleaux in red blood cells (RBCs) with H & E stains and evaluated skin biopsies from diverse ABDs in order

to assess the prevalence of this finding in a small series.

# Methods

### Subjects of study

We examined skin biopsies from patients who fulfilled clinical, histopathological and direct immunofluoresence criteria for ABDs. DIF, in brief, was performed on frozen biopsies kept at minus 20 degrees Celsius that were cut at five micron thickness each. DIF was performed utilizing the antibodies against immunoreactants including IgG, IgA, IgM, IgD, IgE, complement/C1q, complement/C3, complement/C4, Kappa light chains, Lambda light chains, albumin and fibrinogen as previously described [5,6]

We examined 30 biopsies from patients affected by endemic pemphigus foliaceus (EPF) and 20 skin biopsies from normal controls (patients without EPF) from the EPF endemic area (NCEA). We also utilized 30 control skin biopsies from healthy plastic surgery reduction patients, taken from the chest and/ or abdomen normal human skin in the local population (south eastern USA) (NHS). Biopsies were fixed in 10% buffered formalin, then embedded in paraffin and cut at 4 micron thicknesses. The tissue was then stained with H & E. All patients were having primary diagnostic biopsies, and were not taking immunosuppressive therapeutic medications at the time of biopsy. We evaluated 20 biopsies from bullous pemphigoid (BP) patients, 20 from patients with pemphigus vulgaris (PV), eight patient biopsies with pemphigus foliaceus (PF), 12 from patients with dermatitis herpetiformis (DH) and one with linear IgA (LAD). The archival biopsies were IRB exempt due to the lack of patient identifiers.

#### Result

Rouleaux, erythrocytes and some type of autoagglutinitation of RBCs and the presence of a pinkish brush-like material that resembles fibrinoid aggregates were positive in 24/30 biopsies from EPF, in 17/20 patients with BP, in 16/20 in patients with PV, in 10/12 patients with DH, and in the only LAD patient. 30/30 NHS and 15/15 NCEA normal controls skin biopsies failed to demonstrate the finding. The rouleaux, the aggregated RBCs and the pinkish material were uniformly seen under the blisters in most ABDs, and or under the inflamed vessel were close to the blisters (Fig. 1, 2).



**Figure 1 and 2.** Shows representative H & E stain in the different ABDs showing the presence of rouleux, individuals and agglutinned erythrocytes with the pinkish material in the upper dermis close to the blisters black arrows (20X).

### Discussion

In several ABDs, it has been reported that the number of erythrocytes, hematocrit and the amount of hemoglobin decreases in patients with advanced autoimmune bullous diseases, mainly in the pemphigus group [4,7]. Rouleaux are a linear arrangement of red blood cells (RBC) sometimes known as having a "coinstack" configuration. This phenomenon has been associated with increased fibrinogen, globulins, or paraproteins [8-10]. The serum of patients with ABDs is rich in circulating autoantibodies as well as fibrinogen and these are usually deposited in lesional skin in most ABDs patients [3]. Roulex also occur in acute and chronic inflammatory disorders, Waldenstrom's macroglobulinemia and multiple myeloma [2-5]. RBCs in the presence of plasma proteins or other macromolecules may form aggregates, normally in rouleaux formations, which are dispersed with increasing blood flow [8-10].

Based on our findings of rouleaux, the pinkish material, the

dilatation and/or alteration of the vessels proximal to blisters in association with the presence of inflammation, we believe that the formation of rouleax results from flow and permeability alterations. It is also possible that the endothelial cells lose some of their strict regulation due to the adjacent tissue damage, especially beneath the blisters. All these changes may result in the sudden release of inflammatory cells, RBCs, fibronectine, fibrin and other materials that get mixed with some of the acantholytic cells, cells altering that microbalance. The plasma proteins in various ABDs have been described to be altered [11]. This may produce alterations in the blood flow, and may result in rouleaux formation, with or without autoagglutination, clumpled with lysed cells and extravasation of molecules.

Electron microscopy studies demonstrated that in most ABDs the blood vessels show alterations indicative of an outward passage of fluid and the adjacent dermal, and edema separating collagen fibers of the collagen bundles [12,13].

The presence of erythrocytes outside the upper vessels and under and or inside the blister could not be attributed solely to procedural trauma caused by the biopsy. If this were the case we would see them in the periphery of the biopsy and in the cases examined, these findings were in the central portions of the biopsy specimens.

To the best of our knowledge, this is the first report of rouleaux/ and /or autoagglutination in the series of lesional skin biopsies taken from patients with ABDs. The significance of these findings is a topic for a more intense and thorough investigation.

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