

## Auto-antibodies in autoimmune bullous dermatosis

AIBD

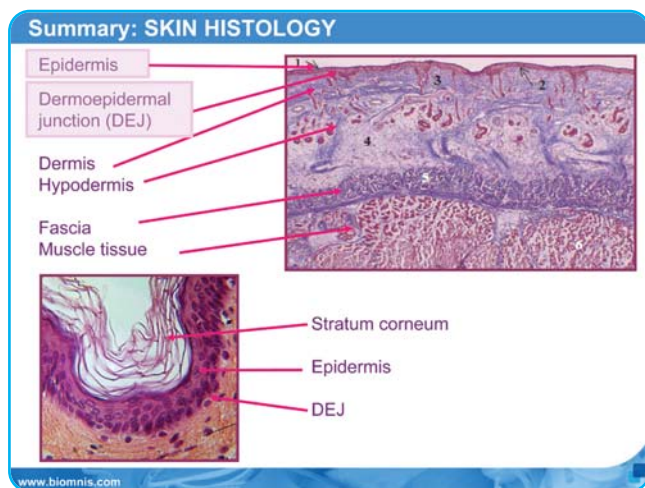
### Definitions

#### Clinical:

characterised by bullous skin (and/or mucosal) lesions associated with the presence of auto-antibodies fixing on the structures providing the cohesion of the epidermis or the dermoepidermal junction. These antibodies are deposited on their target (skin) or are circulating.

According to GOLD STANDARD criteria: the diagnosis of AIBD (autoimmune bullous dermatosis) is based on the combination of clinical, histological and immunopathological criteria. Circulating antibody screening is required in any suspected case of AIBD, as it assists with patient diagnosis and follow-up.

#### Skin histology:



Skin consists, from the outside in, of the epidermis comprised of 80% keratinocytes followed by the dermoepidermal junction (DEJ), dermis and hypodermis.

According to the autoantibody target, a distinction is made between:

- intraepidermal AIBD
- subepidermal AIBD

### Intraepidermal AIBD (pemphigus group):

is characterised by loss of interkeratinocytic cohesion.

A distinction is made between 3 major groups according to the skin or mucosal location of the lesions:

- **Deep pemphigus or pemphigus vulgaris (PV):** rare form, affecting subjects between 40 and 50 years of age, characterised by painful oral mucosal erosions subsequently spreading to the skin.
- **Pemphigus superficialis (PS):** endemic P. erythematosus foliaceus characterised by erythematous-squamous lesions on seborrheic areas but without mucosal lesions. The distinction between (PV) and (PS) is made by means of histology.
- **Paraneoplastic pemphigus (PNP)** associated with some neoplastic, particularly lymphoproliferative, conditions.

**Pemphigus diagnosis** determined on the following 3 criteria:

- **Clinical:** appearance and location of blisters/Nikolsky's sign.
- **Histology:** performed using an intact intraepidermal blister biopsy. The blisters are essentially acantholytic, with a deep cleavage site for PV and superficial site for PS.
- **Direct immunofluorescence:** "latticed" marking of the intrakeratinocytic junction, liable to be associated with linear marking in cases of PNP. The Pemphigus group is characterised by the presence of anti-desmosome antibodies.

### Subepidermal AIBD (pemphigoid) :

Subepidermal AIBD or the Pemphigoid group is characterised by dermoepidermal junction impairment.

A distinction is made between:

- **Bullous pemphigoid:** its frequency is on the rise and affects subjects over 70 years of age, characterised by eczematiform lesions with large blisters.
- **Cicatricial pemphigoid:** affects the mucosa of the eyes (with risk of blindness), mouth and genitals.
- **Gestational pemphigoid** or *herpes gestationis*: a rare form of pemphigoid which is characterised by intense abdominal itching in the first trimester of pregnancy, regresses following delivery but with a risk of recurrence in subsequent pregnancies.
- **Other:** Epidermolysis Bullosa Acquisita (EBA), linear IgA dermatosis, herpetiform dermatitis.

**Pemphigoid diagnosis:**

- **Clinical:** see above
- **Histology:** tight blisters with dermoepidermal detachment are observed.
- **Direct immunofluorescence** displays linear marking along the basement membrane. Subepidermal AIBD is cha-

racterised by the presence of antibodies targeted against dermoepidermal junction (DEJ) antigens.

**IFI in AIBD** is the reference method for screening and quantifying serum antibodies.

- **In Pemphigus**, "latticed" marking of the inter-keratinocytic substance is observed on rat oesophagus and monkey oesophagus, referred to as anti-intercellular substance (anti-ICS) antibodies.
- **In Bullous pemphigoid**: linear marking along the DEJ is observed on rat oesophagus and human skin, referred to as anti-basement membrane (anti-BM) antibodies.

The antigens in the antibodies found in AIBD have been identified by means of various techniques, including WBlot and IME. In this way, the main targets identified in cases of pemphigus are desmosomes (main structures responsible for interkeratinocytic adhesion). They consist of 3 parts, including one extracellular region comprised of desmogleins (Dsg) and desmocollins providing the link between cells. Three isoforms of Dsg exist. Moreover, it has been demonstrated that the primary target antigens of pemphigus V, S and PNP are desmogleins 1 and 3: anti-Dsg 3 for PV and anti-Dsg 1 for PS. In cases of subepidermal AIBD, the target antigens are molecules involved in dermo-epidermal adhesion, comprised of hemidesmosomes and the basement membrane. In the hemidesmosomes located in the basal pole basal keratinocytes, BP 200 protein and the intracellular part of BP 180 protein have been detected. In the basement membrane, the lamina lucida is passed through by anchoring filaments including the external part of BP 180 protein. It has been demonstrated that BP 180 antigen was the target of auto-antibodies in cases of BP and GP.

### ELISA tests in AIBD:

Once the main proteins were identified, recombinant proteins were produced and used to develop ELISA tests. In this way, the antigen used is fixed on a microplate; the patient's serum is deposited, the complexes are antiglobulins labelled with an enzyme and the reaction is detected by colorimetry. Professor Amagai's team in Japan has developed anti-Dsg 1 and Dsg 3 ELISA tests to diagnose Pemphigus by means of target epitopes consisting of the extracellular part of these proteins. The same team has also developed and marketed an ELISA test against the target epitope of BP 180 protein, referred to as region NC16 a.

## Elisa test evaluation for AIBD diagnosis:

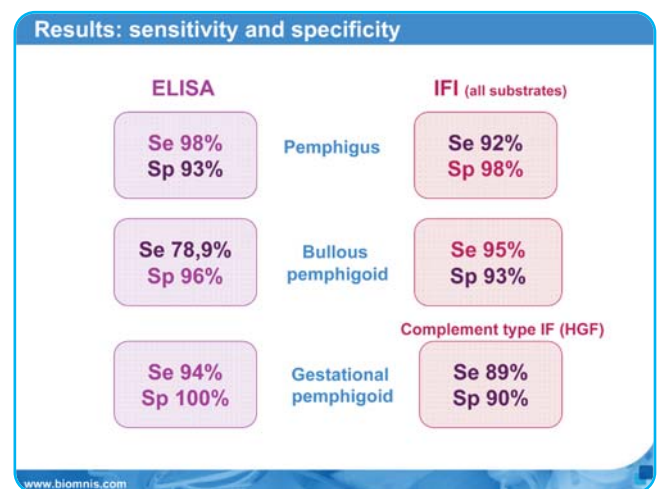
- The study was performed within the scope of the objectives claimed by the bullous disease reference centre in the greater Paris region.
- The purpose of the study was to evaluate commercial anti-Dsg 1, anti-Dsg 3 and anti-BP180 Elisa tests in AIBD diagnostics, particularly in the case of pemphigus: BP and GP. The results obtained with ELISA were compared to the results obtained with IFI on 3 substrates (rat, monkey oesophagus and human skin).
- The enrolment criteria were defined for our various populations: clinical, histology and DIF results of patients whose serum was received and stored in the laboratory between 1993 and 2005. The control population was selected discerningly.
- The question consisted of determining whether ELISA tests can be substituted for IFI in the diagnosis of Pemphigus, BP and GP?

### Materials:

- **48 cases of Pemphigus**: 29 PV, 12 PS, 4 PNP, 3 non-classifiable
- **103 Controls**: 54 non-AIBD, 43 BP, 6 others
- ▶ **Anti-Dsg 1 and anti-Dsg 3**
- **57 cases of Bullous pemphigoid**
- **103 controls**: 54 non-AIBD, 43 Pemphigus, 6 other carriers of other auto-antibodies suspected of causing false positives.
- ▶ **Anti-BP180**
- **18 cases of Gestational pemphigoid**
- **10 controls**: pregnant women
- ▶ **Anti-BP180**

### Results:

The sensitivities and specificities of the ELISA and IFI tests on 3 substrates are shown below :



With IFI, it is necessary to associate at least 2 substrates to obtain a satisfactory sensitivity in AIBD diagnosis.

- **In Pemphigus**, particularly PV and PNP, monkey oesophagus and rat oesophagus are the most sensitive substrates. The study made it possible to demonstrate a correlation between the titre on these substrates (RO and SO) and the anti-Dsg1 index, which is of particular interest in patient follow-up.
- **In cases of Bullous pemphigoid**, the results are very different, the most sensitive substrate is human skin, particularly cleaved human skin, which also makes it possible to make a differential diagnosis between BP and EBA.

## Conclusion:

### Anti-Dsg1 and anti-Dsg3 Elisa:

- Excellent sensitivity and specificity for Pemphigus diagnosis.
- Enables discrimination between PV and PS and could replace IFI when the pemphigus diagnosis is established with positive DIF.

### Anti-BP 180 NCI6a Elisa:

- Insufficient for BP diagnosis, IFI remains the reference technique, particularly on cleaved skin for BP/EBA differential diagnosis.
- May replace complement type IF in GP diagnosis as the technique is easier and more rapid

Elisa is a standardised and automatable method; however, IFI makes it possible to screen for all forms of AIBD at the same time.

