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Serological diagnostics in the detection of IgG autoantibodies against human collagen VII in epidermolysis bullosa acquisita – a multicenter analysis

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Summary

Background: Epidermolysis bullosa acquisita is a rare, potentially devastating autoimmune disease of the skin. IgG autoantibodies directed against type VII collagen (Col7), the major component of anchoring fibrils, induce skin fragility leading to cutaneous and mucocutaneous blister formation which is mostly of a scaring phenotype. Thus, powerful and reproducible diagnostic assays are critical to establish the diagnosis of EBA early to avoid irreversible sequelae.

Objectives: The present international, retrospective multicenter study includes a large cohort of EBA patients and evaluated the diagnostic power of four different diagnostic assays for the detection of anti-Col7 IgG autoantibodies.

Methods: 95 EBA sera and 200 control sera consisting of 100 bullous pemphigoid sera, 50 pemphigus vulgaris sera and 50 sera of healthy controls were tested for anti-Col7 IgG

autoantibodies by indirect immunofluorescence (IIF), two commercial ELISA systems and Western blot (WB) analysis. EBA sera were from patients with positive DIF and IgG reactivity in at least one of the immune serological assays (IIF, ELISA, WB).
Results: A Col7-NC1/NC2 ELISA (MBL, Japan) showed the highest sensitivity (97.9%), followed by a Col7-NC1 ELISA (Euroimmun, Germany; 89.5%), WB with Col7-NC1 (85.3%), and IIF on saline split human skin (74.7%). The specificities of both ELISA systems were comparable (NC1: 98.7%, NC1/NC2: 99.3%). Furthermore, WB was more sensitive than IIF which was more specific.
Conclusions: The two commercially available ELISA systems allow for a highly sensitive and specific diagnosis of EBA. The sensitivity of the Col7-NC1/NC2 ELISA is significantly higher compared to the ELISA which is based on the Col7-NC1-domain only.

What is already known about this topic?

- Diagnosis of EBA is based on positive DIF and the immune serological detection of IgG autoantibodies against type VII collagen (Col7).
- Apart from DIF, skin-bound IgG autoantibodies can be detected at a high sensitivity and specificity by immune electron microscopy and serration pattern analysis of DIF.
- Indirect immunofluorescence, ELISA and Western blotting are mostly commonly used methods for the serological detection of anti-Col7 IgG autoantibodies.

What does this study add?

- The study contains the largest cohort of EBA patients in the context of studies dealing with the serological detection of anti-Col7 IgG autoantibodies.

- The two commercially available ELISA systems showed the highest sensitivity and highest rates of specificity.

- The results demonstrate that the sensitivity of the Col7-NC1/NC2 domain ELISA is significantly higher compared to the ELISA which is based on the Col7-NC1 domain only.

Funding sources

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Conflict of interest

M. Hertl received an unrestricted grant from MBL (Nagoya, Japan). T. Hashimoto had previous collaborations with MBL. M. Sardy received a registration fee and acted as a scientific advisor for MBL but did not have direct influence on the marketing or production activity.

Introduction

Epidermolysis bullosa acquisita (EBA) is a severe, acquired autoimmune bullous disease with a prevalence of approximately 1-5 per million people [1]. It is characterized by the presence of IgG autoantibodies directed against type VII collagen (Col7), a major component of anchoring fibrils, which are responsible for the anchorage of the epidermal basal membrane zone (BMZ) to the dermis [2-4]. Binding of anti-Col7 IgG leads to a destabilization of the BMZ resulting in severe skin fragility and subsequent subepidermal loss of adhesion. Thereby, blisters are induced by a direct antibody impact (mechanobullous type) or by an antibody-induced local inflammation cascade including complement activation and neutrophil recruitment (inflammatory type). EBA was first described by Elliot in 1895 and occurs independently of age, gender or ethnic groups [5]. It is genetically associated with the human leukocyte antigen (HLA) major histocompatibility (MHC) class II allele HLA-DR2, which is also associated with other autoimmune diseases [6-8]. Clinically, EBA presents with cutaneous and mucocutaneous blisters and erosions, which often lead to a scaring phenotype with milia formation and nail loss. Due to the wide spectrum of clinical manifestations of EBA, various differential diagnosis including dystrophic epidermolysis bullosa, bullous pemphigoid (BP), linear IgA dermatosis (LAD) and pemphigus vulgaris (PV), have to be considered (Figure 1). The diagnosis of EBA is based on the clinical phenotype in combination with direct immunofluorescence (DIF), indirect immunofluorescence (IIF) on saline split skin (SSS) and the serological detection of anti-Col7 IgG autoantibodies [9]. The present international retrospective multicenter study compared the specificity and sensitivity of four different standardized serological assays for the detection anti-Col7 IgG, including two commercial ELISA systems, IIF on SSS and Western blotting (WB). The results demonstrate that the ELISA systems delivered the highest sensitivity, followed by IIF and WB. Of note, a Col7-NC1/NC2 ELISA showed a significantly higher sensitivity than a Col7-NC1 ELISA while their specificities were comparable. Furthermore, WB was more sensitive than IIF while IIF showed a higher level of specificity than WB.

Material and Methods

Patients' and control sera

A total of 95 EBA sera were analysed in this retrospective multicenter study. For each serum the centers filled out a study form which included information on the methods used for the immune serological assessment of EBA (Supplementary Table 1), clinical phenotype and treatment. The spectrum of diagnostics included clinical appearance, histology, DIF of perilesional skin, IIF (SSS- IgG reactivity with the dermal portion) ELISA and WB analysis. All of the sera were from EBA patients with positive DIF and were positive in at least one serological test (IIF/ELISA/WB). A total of 97.8% of the tested EBA sera were initially tested by IIF (SSS and/or monkey esophagus) in the contributing centers. 3.3% of the sera were tested by ELISA only. Due to the limited relevance of monkey esophagus analysis, the monkey esophagus data were deleted. Thus, 91/95 (95.7%) of the EBA sera were initially tested by SSS in the contributing centers. Of these 91 sera, 3 (3.3%) sera were tested negative. As shown in Supplementary Table 1, 96.7% of the SSS tested EBA sera were initially positive while only 74.7% were positive upon re-analysis in the present study (including four samples which were not initially tested in the respective centers). The observed difference in the sensitivity of SSS analysis of the EBA sera may be related to factors dependent on the time period between initial analysis at the contributing center and our analysis such as storage of the sera which becomes apparent with the SSS as the method of lowest sensitivity compared to WB and ELISA. In contrast, all the EBA sera which were initially tested positive by ELISA in the contributing centers remained positive upon re-analysis in our laboratory. Noteworthy, all of the 6 EBA sera that were initially tested by SSS only in the contributing centers were also positive by SSS upon re-analysis in our laboratory.

Overall, 41% of the sera were collected from European EBA patients (Italy, Germany, Croatia, Turkey, Czech Republic) and 51% from Japanese EBA patients. Noteworthy, 16 of the 48 Japanese EBA sera had been already included in a previous study by Komorowski et al.[10]. The controls included 100 sera from patients with BP, 50 sera from PV patients and 50 from healthy controls. BP and PV were diagnosed by clinical phenotype, DIF of perilesional skin and the serological detection of IgG against bullous pemphigoid antigen 180 (BP180) / BP230 or desmoglein (Dsg1 /Dsg3, respectively. This study was conducted in accordance with the Declaration of Helsinki Guidelines and approved by the local Ethics Committee of the Medical Faculty of the University of Marburg, Germany.

Immunofluorescence microscopy on saline split human skin

Healthy human skin was incubated in a 1M NaCl solution for 48-72h. The skin specimens were then washed three times in PBS for 15 min, embedded in OCT Tissue Tec (Sakure Finetek, Staufen, Germany) and stored at -80°C. After the preparation of microscope slides (Superfrost Plus, Langenbrinck, Emmendingen, Germany) with cryo sections (Microtom Micron HM-560), EBA and control sera were incubated 1:50 (in PBS) with the human skin

cryo sections for 30 min at RT under humid conditions. Sera of BP and PV patients and of healthy individuals served as controls. The slides were washed twice with PBS and incubated for another 30 min with a FITC-conjugated AffiniPure F(ab´)$_2$ fragment rabbit anti-human IgG antibody (Dianova, Hamburg, Germany) under humid conditions in the dark. Subsequently, slides were washed twice again with PBS and the slides were fixed with mounting medium (Fluorescence Mounting Medium, Dako, Jena, Germany) and coverslips were added. The final analysis was done with a fluorescence microscope (Axiostar, Zeiss, Jena, Germany).

SDS Gel Electrophoresis and Western Blotting

The recombinant fragments of human Col7 NC1(1) (aa17-aa610), NC1(2) (aa273-857) and NC1(3) (aa611-1253) were produced in a baculovirus-based eukaryotic expression system as previously described [11]. For gel electrophoresis, equal amounts of Col7-NC1 proteins were denaturated by Laemmli buffer + β-Mercaptoethanol (95°C, 5min) and separated on a 10% SDS-PAGE gel. The WB procedure was performed as previously described [12]. Patient sera were diluted 1:200 in PBST (1 x PBS + 0.1% Tween-20) and incubated overnight by 4°C under shaking conditions. A positive-tested EBA serum (1:200) and a rabbit anti-E-Tag primary antibody (Abcam, Cambrigde, UK) served as positive, a serum from a healthy donor as negative control. Analysis was done by a digital chemoluminiscence reader (PEQLAB, Erlangen, Germany).

Collagen VII ELISA

The NC1 ELISA (Anti-Type VII Collagen-ELISA (IgG), Euroimmun, Lübeck, Germany) and the NC1/NC2 ELISA (MESACUP Anti-Type VII Collagen Test, MBL, Nagoya, Japan) were performed according to manufacturers` protocol. Optical densities were measured at a wavelength of 450 nm and a reference of 620 nm by photometer Sunrise™-Basic (Tecan, Göding, Austria). Calculation of RE/ml (Euroimmun ELISA) and U/ml (MBL ELISA) was done according to the manufacturers' instructions. Samples showing values greater or equal to 20 RE/ml (Euroimmun ELISA) or 6 U/ml (MBL ELISA), respectively, were considered as positive.

Statistics

For each of the four tests (Col7-NC1 ELISA, Col7-NC1/NC2 ELISA, SSS and WB), we calculated estimates and 95% confidence intervals (95% CI) of sensitivity for EBA, BP and PV as well as specificity with respect to healthy control sera, BP/PV controls and the combination of both control groups. We used the method detailed by Tango[13] to compare the sensitivities and specificities, respectively, of Col7-NC1 ELISA, Col7-NC1/NC2 ELISA as well

as with the SSS and WB. To explore cut-off values other than those provided by the manufacturers, ROC curves were calculated for the two ELISA using the RE/ml (Euroimmun ELISA) and U/ml (MBL ELISA) results comparing sensitivity for EBA and specificity with respect to each of the control groups used. All statistical analyses were performed using the R program for statistical computing (R foundation for Statistical Computing, Vienna, Austria).

Results

Sensitivity of diagnostic assays

Four diagnostic assays were employed to detect anti-Col7 IgG in the sera of patients with EBA. These included IIF with the standard substrate, SSS, an in-house WB with the recombinant human CO7-NC1 domain and two commercial ELISA systems with the human Col7-NC1 and Col7-NC1/NC2 domains, respectively. Tested were a total of 95 EBA sera, 100 BP, 50 PV and 50 healthy control sera. The highest sensitivity was obtained with the NC1 ELISA (89.5%) and the NC1/NC2 ELISA (97.9%) followed by WB (85.3%) and IIF with SSS (74.7%). Of note, the NC1/NC2 ELISA exhibited a significantly higher sensitivity compared to the NC1 ELISA (p=0.005) (Table 1). Specifically, in the analysis of the cohort of 95 EBA patients, the NC1/NC2 ELISA delivered 93 positive results and the NC1 ELISA 85 positive results (Figure 4). WB with human Col7-NC1 revealed a significantly higher sensitivity rate than IIF on SSS (p=0.033), whereas the NC1 ELISA and WB results showed no statistical difference regarding sensitivity (p=0.346) (Table 1). Two sera were tested positive by WB or IIF only. Surprisingly, all the six additionally positive test results of the NC1/NC2 ELISA originated from European EBA sera (n=3, Italy, n=2, Germany, n=1, Czech Republic), which remained below the cut-off value of the NC1 ELISA (cut-off level 20 RE/ml).

Specificity of diagnostic assays

The specificity of the diagnostic assays was calculated based on the analysis of 50 healthy control sera, 50 PV and 100 BP sera. Overall, the specificity was high (i.e. >90%) for all tested methods (Figure 3B). The WB delivered a specificity of 94%, IIF with SSS of 99%, and the two ELISA systems of 99% - 100%. The NC1 ELISA showed false positive results in one PV and one BP patient (Figure 4A) while the NC1/NC2 ELISA was false-positive in one BP patient (Figure 4C). By WB, three healthy control sera, six BP and three PV sera were considered to be positive. Hence, the healthy control sera showed the lowest number of false-positive results (n=3) and the WB revealed a significantly lower overall specificity

compared to ELISA and IIF (NC1 ELISA p=0.004; NC1/NC2 ELISA p=0.002; IIF p=0.001) (Table 2).

Diagnostic and predictive power of Col7 ELISA assays

ROC analysis (area under the curve/AUC) revealed no statistical differences between the NC1 ELISA (AUC healthy controls: 0.983 / AUC BP + PV: 0.987) and the NC1/NC2 ELISA (AUC healthy controls: 0.995 / AUC BP + PV: 0.995) (Figure 4A and D)). We also tried to correlate anti-Col7 IgG autoantibodies with the clinical course of selected EBA patients. In patient 1, as expected, immune adsorption led to a strong decrease of anti-Col7 serum IgG levels (T2) but in the course of disease, anti-Col7 serum IgG increased again (T3) and was associated with a worsening of the clinical status. As illustrated by ABSIS score and clinical pictures, anti-Col7 IgG correlated well with disease activity (Figure 5A). In contrast, in patient 2, an increase of anti-Col7 IgG was associated with a slight improvement of skin involvement (TP1 >TP2). Noteworthy, in this patient antibody titers were very consistent on a high level over time (TP2 > TP6 / Figure 5B). In patient 3, complete clinical remission was accompanied by a strong decrease of anti-Col7 serum IgG (Figure 5C). In all three EBA patients, changes in anti-Col7 serum IgG concentrations measured by optical density (OD) were comparable between the Col7-NC1 and Col7-NC1/NC2 ELISAs but ran at different levels (Figure 5 A-C). Overall, anti-Col7 IgG serum concentrations were largely correlated with the clinical disease activity (Figure 5A-C).

Discussion

In this international retrospective multicenter study, we investigated the so far largest cohort of European and Japanese EBA patients to validate the diagnostic power of serological assays designed to detect IgG autoantibodies against Col7, the autoantigen of EBA. We here show that two commercially available human Col7-NC1 ELISAs showed the highest sensitivity among the tested *in vitro* diagnostic assays and were superior to IIF with human SSS and to a WB with overlapping fragments that span the NC1 subdomain of Col7. Moreover, one of the two Col7 ELISAs, which additionally contained the NC2 domain of Col7, showed the highest sensitivity and was thus superior to the Col7-NC1 ELISA.

EBA is a potentially devastating, chronic-relapsing autoimmune bullous disorder of the skin and mucous membranes which is characterized by a pronounced and persistent fragility of the skin leading to blisters and erosions with a scarring phenotype [5]. The therapeutic mainstay of EBA are corticosteroids, which are frequently combined with immunosuppressive drugs such as azathioprine, mycophenolate mofetil, methotrexate or cyclophosphamide [14,15]. Novel anti-inflammatory therapies like high-dose intravenous immunoglobulins (IVIG), the anti-CD20 monoclonal antibody, rituximab, and immunoadsorption (IA) have been employed in EBA with variable success [14,16-18]. The inflammatory type of EBA that clinically resembles BP, responds well to immunosuppressive drugs. In contrast, mechano-bullous EBA is largely refractory to immunosuppressive treatment. In addition, chronic immunosuppressive treatment is associated with major adverse effects [15,19]. Thus, early diagnosis is essential for the optimal therapeutic and functional management of EBA. Central to the proper diagnosis of the different clinical EBA variants is the detection of IgG autoantibodies directed against Col7, which play an essential pathogenic role [4,10,11,16,20-29] (Table 3). In general, anti-Col7 IgG serum concentrations correlate with the disease status [30,31] which is also reflected by the longitudinal analyses of three selected EBA patients of the present study (Figure 5). Currently, in addition to the clinical appearance (which may be quite heterogeneous), the diagnosis of EBA is established based on the detection of tissue-bound (by DIF) and serum anti-Col7 IgG antibodies (by IIF, WB or ELISA). It has been estimated that 50%-80% of EBA patients exhibit both, tissue-bound and circulating anti-Col7 autoantibodies [32]. The gold standard technique for the detection of tissue-bound IgG is immunoelectron microscopy (IEM) which provides the highest sensitivity [33]. Recently, Terra et al. developed a more refined and highly sensitive DIF analysis of EBA which is based on the detection of an u-serrated IgG deposition pattern at the dermal-epidermal BMZ [29]. At present, the overall significance of both, IEM and DIF serration pattern analysis is, however, limited as only a few laboratories worldwide provide this diagnostic technique for EBA. Thus, in the current clinical practice, DIF has proven to be a highly reproducible and robust technique and is considered as reference method for detection of tissue-bound IgG autoantibodies in EBA [34-36]. Thus, all the published studies on the sensitivity and specificity of immunoserological assays in EBA relate to DIF as the gold standard. Its diagnostic power, however, may be overestimated as DIF is less sensitive than IEM and DIF serration pattern analysis [33]. This is reflected by studies of Seta et al. [28] and Terra et al.[29] where a positive IEM or serration pattern result served as an inclusion criterion for the assessment of the diagnosis of EBA. In these studies, the sensitivity of the NC1/NC2 ELISA was much lower (30%/54%) compared to serological studies which defined classical DIF as the gold standard.

The present study clearly shows that WB with human Col7 and IIF with the standard substrate, SSS, are less sensitive than the commercial Col7 ELISAs. Nevertheless, SSS is a

critical technique for the immune serological distinction between BP (epidermal IgG reactivity) and EBA, laminin gamma1 pemphigoid (all dermal IgG reactivity) and mucous membrane pemphigoid (dermal and/or epidermal IgG reactivity) [37]. However, lack of serum IgG reactivity with SSS does not exclude the diagnosis of EBA and further immune serological diagnostics are warranted in case of clinical suspicion of EBA. Many laboratories perform IIF on SSS first before further defining the specificity of anti-dermal epidermal IgG autoantibodies by ELISA. The SSS sensitivity rate of 74.7% of the present study is comparable to previous studies by Chen et al. (78%) [21] and Calabresi et al. (83.3%) [20]. However, there may be an overestimation of the diagnostic power of IIF by SSS as several serological studies in EBA defined IIF positivity as one of the inclusion criteria. The present retrospective study showed that longterm storage of EBA sera diminished their reactivity with SSS as 96.7% of the SSS tested EBA sera were initially positive but only 74.7% were positive upon re-analysis. In contrast, Col7 ELISA reactivity was not affected by storage of the sera. This potential technical bias in evaluating the different serological techniques should be addressed in a prospective trial.

The sensitivity of WB with Col7-NC1 (85.3 %) was similar to a previous study by Saleh et al. (89.8% / based on dermal extracts), who also found a significantly higher sensitivity of the Col7-NC1 ELISA [27]. In the case of WB, due to the technical variations, a reliable comparison of the methods is hardly possible. Overall, due to its limited diagnostic power, Col7 WB analysis is of limited value in EBA.

Col7, a typical collagenous protein, is a homotrimer consisting of three α-chains, which form a triple helical collagenous structure. Each of the trimers contains a NH2-terminal (NC1) and a COOH-terminal non-collagenous domain (NC2) [38]. Most of the EBA patients exhibit serum IgG autoantibodies against the immunodominant NC1 domain of Col7 [39]. In addition, several reports have identified Col7-NC2-specific IgG autoantibodies in EBA [40-45]. Saleh et al. detected Col7-NC2-specific serum IgG in 16.2% of the studied EBA sera (n=49) [27]. Noteworthy, in this study, 2% of the EBA patients exclusively showed IgG serum reactivity against Col7-NC2. These findings explain why the overall sensitivity of the Col7-NC1/NC2 ELISA (MBL, Japan) is superior to the Col7-NC1 ELISA (Euroimmun, Germany). Of note, most of the previous studies investigating the performance of the Col7-NC1 and Col7-NC1/NC2 ELISA, uniformly found a very high specificity but variable sensitivity rates. Our findings are in line with a previous study by Komorowski et al. [10], who found similar rates of sensitivity (91.8%) and specificity (98.7%) in their cohort of 73 EBA patients (Col7-NC1 ELISA, Euroimmun, Germany). Thereby, in multicenter studies the described sensitivities are dependent of the diagnostic methods performed in the participating centers to establish the diagnosis of EBA.

In summary, this so far largest analysis of European and Japanese EBA sera demonstrates that two commercial ELISA systems based on recombinant human Col7-NC1 deliver the highest sensitivity and specificity for the detection of anti-Col7-IgG compared to IIF with the standard substrate, SSS, and WB with human Col7-NC1. Furthermore, our findings clearly show that supplementation of the Col7-NC1 ELISA with the human Col-NC2 subdomain leads to a significant increase of the diagnostic power of the Col7 ELISA.

FIGURE LEGENDS

Fig. 1: Clinical differential diagnosis of autoimmune bullous skin disorders. A, epidermolysis bullosa aquisita (skin); B, bullous pemphigoid; C, laminin-γ1 pemphigoid; D, epidermolysis bullosa acquisita (oral mucosa); E, pemphigus vulgaris (oral mucosa).

Fig. 2: Synopsis of diagnostic assays utilizing distinct subdomains of collagen VII (Col7).

Fig. 3: Sensitivity and specificity of diagnostic assays utilizing collagen VII (Col7) in epidermolysis bullosa acquisita. A, Sensitivity rates of salt split skin (SSS), NC1-Western blot (WB) with Col7-NC1, Col7-NC1-ELISA and Co7-NC1/NC2 ELISA; B, Specificity rates of SSS, WB, NC1-ELISA and NC1/NC2-ELISA.

Fig. 4: Performance of two ELISA systems in epidermolysis bullosa acquisita. A, Combined box-and-whisker plots showing collagen VII (Col7) NC1-ELISA results. B, ROC analysis of Col7-NC1-ELISA results. C, Combined box-and-whisker plots showing Col7-NC1/NC2-ELISA results. B, ROC analysis of Col7-NC1/NC2-ELISA results.

Fig. 5: Longitudinal analysis of anti-collagen VII (Col7) IgG serum concentrations and clinical course of patients with epidermolysis bullosa acquisita (EBA). A, Patient 1: Autoimmune Bullous Skin Disorder (ABSIS) score, anti-Col7 IgG by ELISA (Col7-NC1 : RE/ ml ; Col7-NC1/NC2 : U/ ml ; IA=immunoadsorption) and illustration of clinical symptoms (T4, T5, T6 and T7), . B, Patient 2: Anti-Col7 IgG by ELISA (Col7-NC1 : RE/ ml ; Col7-NC1/NC2 : U/ ml) and description of clinical symptoms. C, Patient 3 : Anti-Col7 IgG by ELISA (Col7-NC1 : RE/ ml ; Col7-NC1/NC2 : U/ ml) and description of clinical phenotype.

Table 1: Sensitivity of diagnostic assays in epidermolysis bullosa acquisita

p values (95% CI)	SSS	WB	NC1 ELISA	NC1/NC2 ELISA
SSS		0.033 (-0.205<>-0.009)	0.002 (0.061<>0.243)	0.000 (0.148<>0.329)
WB	0.033 (-0.205<>-0.009)		0.346 (-0.049<>0.136)	0.001 (0.059<>0.212)
NC1 ELISA	0.002 (0.061<>0.243)	0.346 (-0.049<>0.136)		0.005 (0.042<>0.157)
NC1/NC2 ELISA	0.000 (0.148<>0.329)	0.001 (0.059<>0.212)	0.005 (0.042<>0.157)	

Table 2: Specificity of diagnostic assays in epidermolysis bullosa acquisita

p values (95% CI)	SSS	WB	NC1 ELISA	NC1/NC2 ELISA
SSS		0.001 (0.035<>0.102)	0.157 (-0.036<>0.009	0.317 (-0.028<>0.014)
WB	0.001 (0.035<>0.102)		0.004 (0.02<>0.092)	0.002 (0.024<>0.098)
NC1 ELISA	0.157 (-0.036<>0.009	0.004 (0.02<>0.092)		0.564 (-0.019<>0.031
NC1/NC2 ELISA	0.317 (-0.028<>0.014)	0.002 (0.024<>0.098)	0.564 (-0.019<>0.031)	

Table 3: Sensitivity and specificity of diagnostic assays in epidermolysis bullosa acquisita

				Sensitivity %			Specificity %	
Authors	Year	EBA sera	Control sera	SSS	WB	ELISA	ELISA	Recombinant protein
Chen et al.	1997	24	39	78	71.4	100	100	Col7-NC1
Chen et al.	2007	32	27	---	66.6	83.3	100	Col7-NC1 (aa1-227)
Pendaries et al.	2009	41	55	---	---	68	96	Full length-C7 ELISA
Mueller et al.	2010	15	50	93.5	73	66.6 (combination of all fragments)	100 (combination of all fragments)	Col7-NC1 peptides (aa17-610, aa273-857, aa611-1253)
Saleh et al.	2011	49	95	100	89.8	93.8 (combination of two fragments)	98.1 (combination of two fragments)	Col7-NC1 Col7-NC2
Marzano et al.	2013	14	143	100	93	85.7 (combination of two fragments)	98.1 (combination of two fragments)	Col7-NC1 Col7-NC2
Licarete et al.	2012	50	363	---	---	94 (combination of all fragments)	97.3 (combination of all fragments)	Col7-NC1 Col7-NC2 Col7- hinge region
Kim et al.	2012	30	53	---	---	96.7	98.1	Commercial ELISA# (Col7-NC1-NC2)
Komorowski et al.	2012	73	389	99.8	91.8	91.8	98.7	Col7-NC1
Terra et al.	2013	28	46	57.1	---	54	97.8	Commercial ELISA# (Col7-NC1-NC2)
Calabresi et al.	2014	24	85	83.3	91.7	79.2	98.8	Commercial ELISA# (Col7-NC1-NC2)
Horvath et al.	2016	21	40	100	---	81	100	Commercial ELISA# (Col7-NC1-NC2)
Seta et al.	2016	77	67	27	---	65	97	Full length-

						(30)	(96)	C7 ELISA Commercial ELISA# (Col7-NC1-NC2)
Marzano et al.	2016	6	11	100	---	100	100	Commercial ELISA# (Col7-NC1-NC2)
Present study	2017	95	200	74.74	85.26	89.47	98.7	Commercial ELISA* (Col7-NC1)
Present study	2017	95	200	74.74	85.26	97.87	99.3	Commercial ELISA# (Col7-NC1-NC2)

*Manufacturer: Euroimmun, Lübeck, Germany (Col7-NC1: aa1-1253)

#Manufacturer: MBL, Nagoya, Japan (Col7-NC1: aa21-1253, NC2: aa2783-2944)

S Table 1: Serological diagnostics to establish the diagnosis of epidermolysis bullosa acquisita by the contributing clinical centers (n=95).

SSS	ELISA	Immunoblot	% of tested sera
+	+	+	71.5%
+	+	-	11.6%
+	-	+	6.3%
+	-	-	6.3%
-	+	-	3.3%
-	+	+	1.1%
-	-	+	0%
91/95 sera	83/95 sera	75/95 sera	

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Figure 1

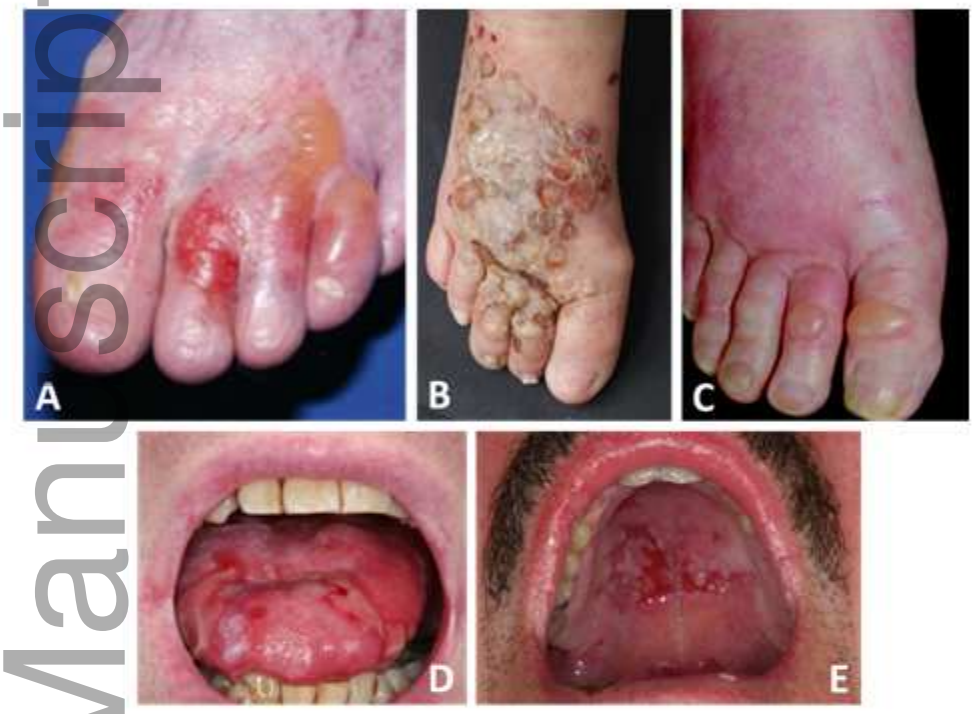
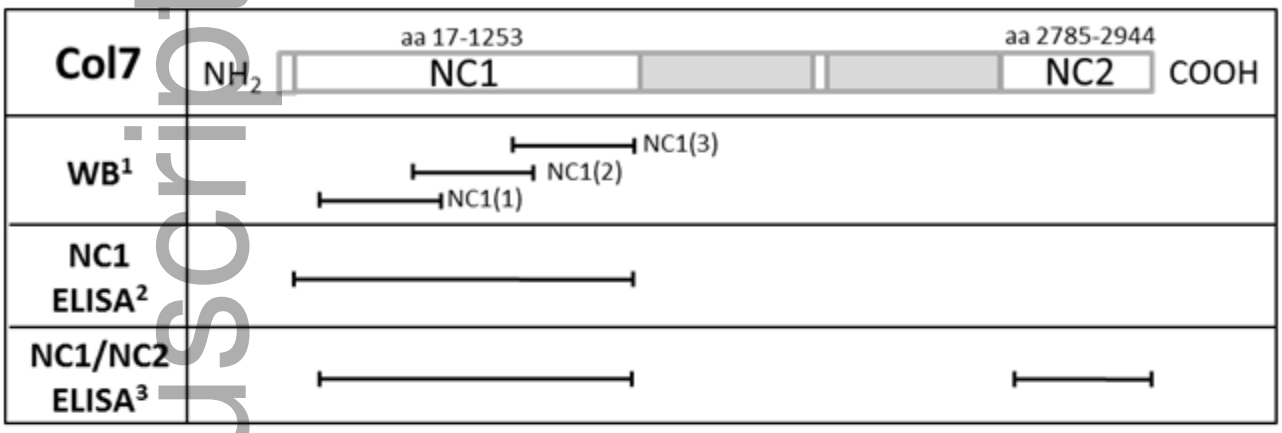


Figure 2



[1]NC1(1): aa17-610, NC1(2): aa273-857, NC1(3): aa611-1253
[2]NC1: aa1-aa1253
[3]NC1: aa21-aa1253; NC2: aa2783-aa2944

Figure 3

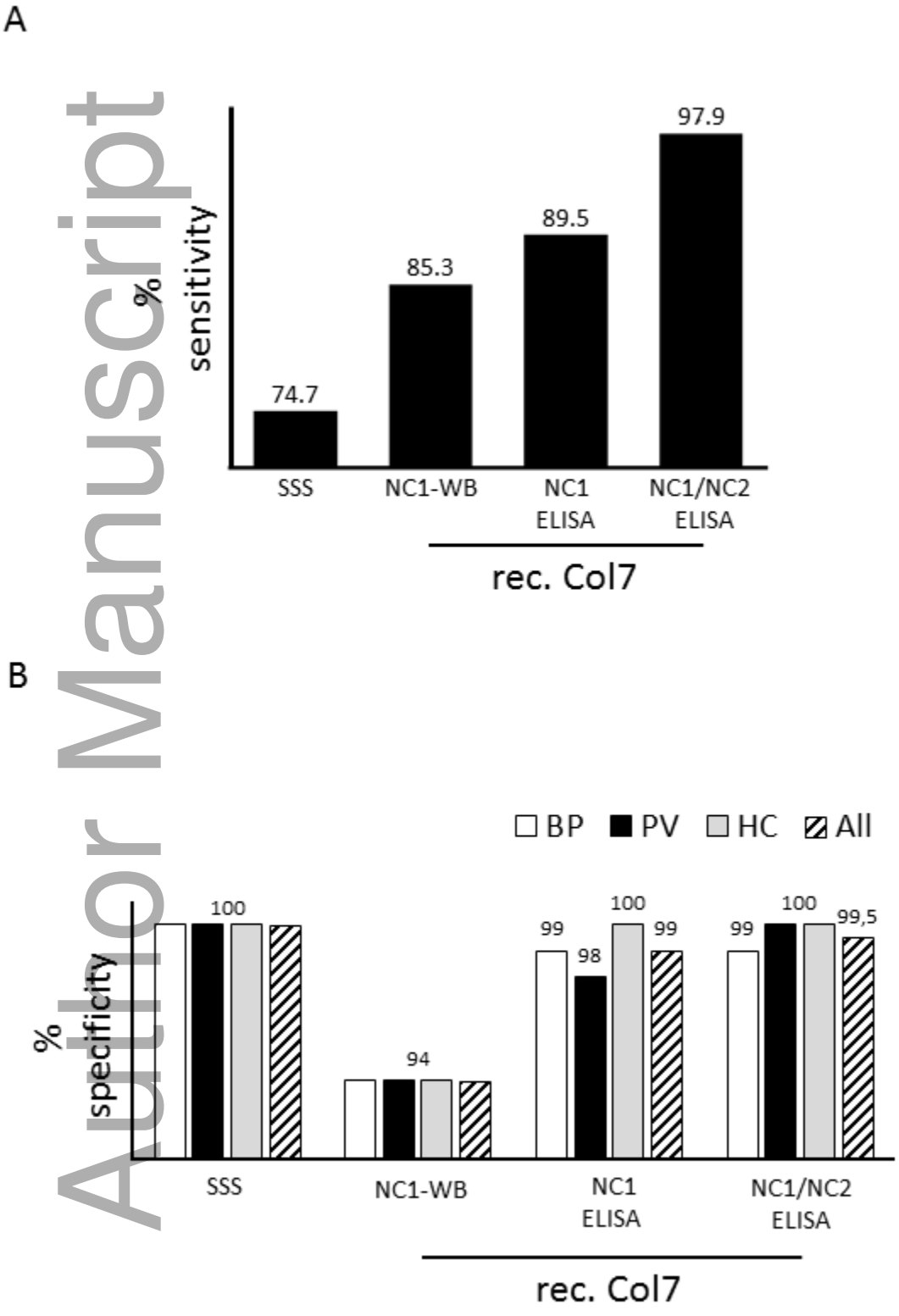


Figure 4

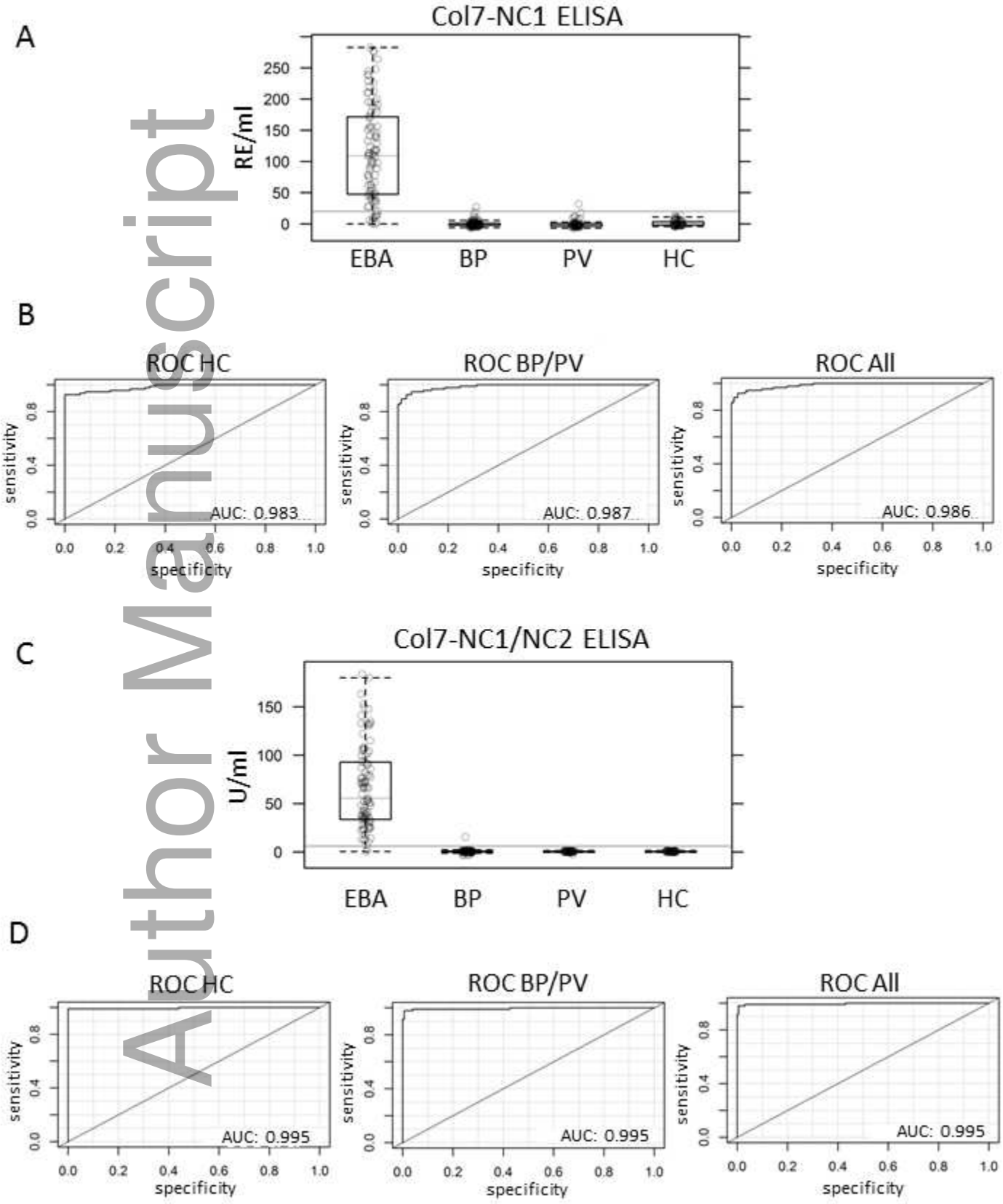
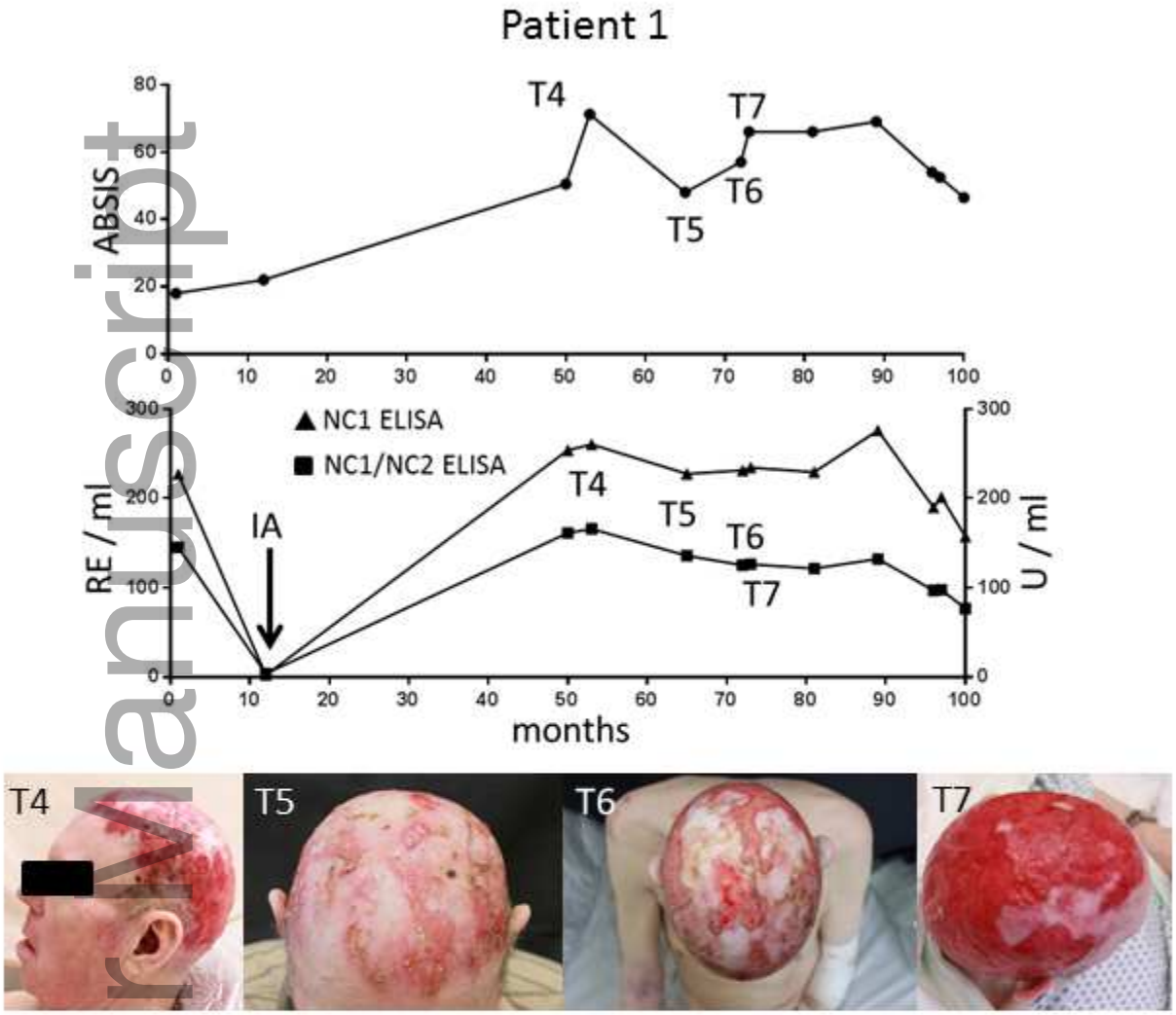
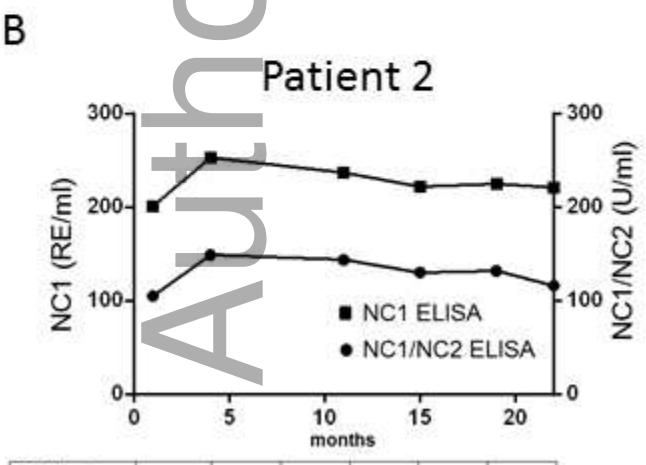
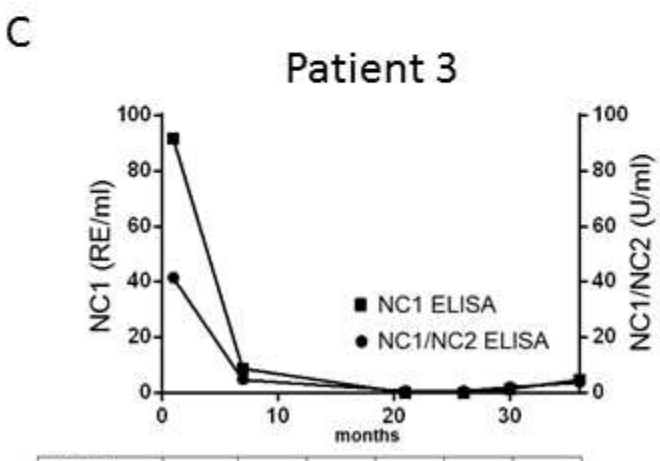


Figure 5





Clinical phenotype	T1	T2	T3	T4	T5	T6
Bullae	+	-	+	-	-	-
Erosions	+	+	+	+	+	+
Erythema	+	-	+	+	+	+
Itch	+	+	+	+	+	+



Clinical phenotype	T1	T2	T3	T4	T5	T6
Bullae	-	-	-	-	-	-
Erosions	+	+	-	-	-	-
Erythema	+	-	-	-	-	-
Itch	+	-	-	-	-	-

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