Received: 30 March 2023 | Accepted: 17 July 2023

DOI: 10.1111/jdv.19424

JEADV JOURNAL OF THE EUROPEAN ACADEMY OF DERMATOLOGY & VENEREOLOGY

SHORT REPORT

# Fast, bedside diagnosis of toxic epidermal necrolysis using ex vivo confocal laser scanning microscopy: A retrospective study

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**Abstract**

**Background:** Toxic epidermal necrolysis (TEN) is a severe life-threatening drug eruption with rapid evolution. A fast histologic differentiation between TEN and clinically similarly looking staphylococcal scalded skin syndrome is of vital importance for relevant treatment decision. The recently developed ex vivo confocal laser scanning microscopy (CLSM) offers innovative and extremely fast histological visualization of fresh tissue specimens.

**Objective:** To assess the diagnostic efficacy of ex vivo CLSM in comparison with standard histopathology for TEN.

**Methods:** We performed side-by-side comparison of TEN specimens analysed with ex vivo CLSM and haematoxylin and eosin staining. Analysis focused on typical histopathological features of TEN, including epidermal cleavage in the basal layer and confluent epidermal necrosis. We retrospectively assessed the diagnostic performance of ex vivo CLSM for TEN in clinically confirmed cases.

**Results:** We report substantial agreement between ex vivo CLSM and classical histology for the detection of subepidermal cleavage and confluent epidermal necrosis. When considering full-thickness epidermal loss, epidermal cleavage in the basal layer showed the highest diagnostic performance, reaching 87.5% sensitivity and 100% specificity.

**Conclusion:** Based on our data, ex vivo CSLM appears as a rapid, resource-optimizing, and reliable approach for morphological TEN emergency screening on fresh skin samples.

## INTRODUCTION

Toxic epidermal necrolysis (TEN) is a rare but life-threatening, adverse drug reaction, characterized by widespread, full-thickness necrosis of the epidermis.[1] Since TEN has a high mortality rate (30%) and long-term morbidity,[2] early diagnosis is critical for rapid identification and cessation of the culprit drug, and supportive care in a specialized intensive care unit. While TEN patients present typical clinical features, histopathological skin examination is essential to support the diagnosis. Indeed, current international consortia recommend fresh-frozen sectioning of skin specimens for fast histopathological analysis of suspected TEN, looking for full-thickness epidermal necrosis and/or epidermal cleavage in the basal layer.[3] In particular, staphylococcal scalded skin syndrome (SSSS) can present with erythroderma and superficial epidermal peeling, mimicking TEN clinically. However, in contrast to the epidermal cleavage in the basal layer characteristic for TEN, the cleavage induced by Staphylococcus aureus exotoxins is situated at the subcorneal level.[4] Histopathological analysis is thus very efficient in distinguishing TEN from SSSS, and as the quick delineation of TEN from SSSS is of vital clinical importance, frozen sections are the current gold standard for bedside screening.[5]

Ex vivo confocal laser scanning microscopy (CLSM) has recently emerged as a technology allowing high-resolution images of fresh tissues, at cellular level, with good correlation when compared to conventional H&E histopathology

in various skin diseases,[6] sparing the time-consuming and costly procedure of tissue fixation, cutting and staining. We thus aimed to study the diagnostic efficacy of ex vivo CLSM in comparison with conventional H&E histopathological procedures for TEN.

## METHODS

### Setting

We retrospectively analysed 21 frozen skin samples, of which 8 TENs, 1 SSSS, 6 severe maculo-papular drug eruptions and 6 healthy skin at Lausanne University Hospital. The procedure followed was in accordance with Helsinki Declaration of 1975, as revised in 1983 and approved by the institutional review board of Lausanne University Hospital CHUV, and the local ethics committee (study 2015-00187). Each patient enrolled provided written informed consent. Skin biopsies were defrost, washed in phosphate buffered saline (PBS) solution, rinsed in 70% ethanol and submerged in acridine orange and indocyanine green. After final PBS washing, 1024 × 1024 pixels images (single field of view: 550 × 550 µm, maximum mosaic area: 25 × 25 mm) were acquired with 488 nm (blue, fluorescence) and 638 nm (red, reflection) wavelengths on VivaScope 2500-G4® (Mavig GmbH). Digital H&E-like staining and reflectance modes were used for the examination of the general morphology and disease-related pathologic features. Confocal digital images were evaluated by LT, FK, EG and MV in a blinded fashion. H&E staining from paraffin-embedded parallel samples were evaluated by independent, blinded dermatopathologists. The following parameters were reported: epidermal cleavage in the basal layer, confluent epidermal necrosis and full-thickness epidermal loss.

### Statistics

We assessed the consistency of ex vivo CLSM and conventional histopathological H&E staining for the detection of histopathological features of TEN. Therefore, we performed a Fleiss' kappa test, in which the level of agreement was classified as less than by chance ($\kappa < 0$), slight ($\kappa < 0.20$), fair ($\kappa = 0.21–0.40$), moderate ($\kappa = 0.41–0.60$), substantial ($\kappa = 0.61–0.80$), almost perfect ($\kappa = 0.81–0.99$) and perfect ($\kappa = 1$). We determined the sensitivity and specificity of single or combined histomorphological features related to TEN and seen in ex vivo CLSM by comparing TEN and control samples. *p*-values were calculated using a Student *t*-test.

## RESULTS

A total of 8 skin samples from patients with clinical and histological diagnosis of TEN and 13 control samples were examined using ex vivo CLSM. Control group included 1 SSSS, 6 severe maculo-papular drug eruptions and 6 healthy skin specimens. Digital H&E-like staining and reflectance mode were overlaid for the examination of TEN-related histomorphological features. Ex vivo CLSM identified epidermal cleavage in the basal layer in 62.5% ($n = 5$), confluent epidermal necrosis in 62.5% ($n = 5$) and full-thickness loss in 25% ($n = 2$) of TEN specimens (Figure 1a). The agreement level between ex vivo CLSM and conventional H&E histology was substantial for confluent epidermal necrosis and epidermal cleavage in the basal layer, while perfect for full-thickness epidermal loss (Table 1). We then compared TEN skin specimens to the above-mentioned control samples using ex vivo CLSM (Figure 1a–c). We found significant diagnostic efficacy using either epidermal cleavage in the basal layer (62.5% sensitivity and 100% specificity) or confluent epidermal necrosis (62.5% sensitivity and 22.2% specificity) as discriminative criteria (Table 2). Remarkably, when considering full-thickness loss together with epidermal cleavage in the basal layer, the diagnostic performance of ex vivo CLSM reached 87.5% sensitivity and 100% specificity (Table 2), with almost perfect level of agreement between 3 independent, blinded evaluations (calculated Fleiss' kappa 0.93; $p < 0.001$).

## DISCUSSION

In this study, we evaluate the applicability of ex vivo CLSM for the ex tempore diagnosis of TEN. Ex vivo CLSM allows real-time, ultra-rapid imaging, without the need of fixing, embedding, sectioning and staining as required for conventional histopathology. As such, it seems particularly suited for fast decision-making when suspecting TEN, avoiding the need for 24/24 technical assistance and allowing possible use of telemedicine (dermato-pathologist in remote). Importantly, we identify representative morphological features of TEN using ex vivo CLSM, such as confluent epidermal necrosis and epidermal cleavage in the basal layer, with satisfactory agreement compared to conventional H&E histology. We show that ex vivo CLSM-mediated detection of epidermal cleavage in the basal layer has the strongest diagnostic performance, reaching 87.5% sensitivity with 100% specificity (considering full-thickness epidermal loss). This is particularly important in the clinical setting, as fast, bedside exclusion of SSSS is critical for an appropriate therapeutic care. Here, we show that the epidermal cleavage in the basal layer seen by CLSM in TEN is not found in severe maculo-papular drug-induced eruptions (Figure 1b) and is very well distinguished from the subcorneal cleavage seen in SSSS (Figure 1c).

### Limitations

CLSM may not identify early TEN patients where histopathological features may be more subtle. Similarly, it may hardly discriminate TEN from skin diseases presenting with large epidermal detachment in the basal layer, like

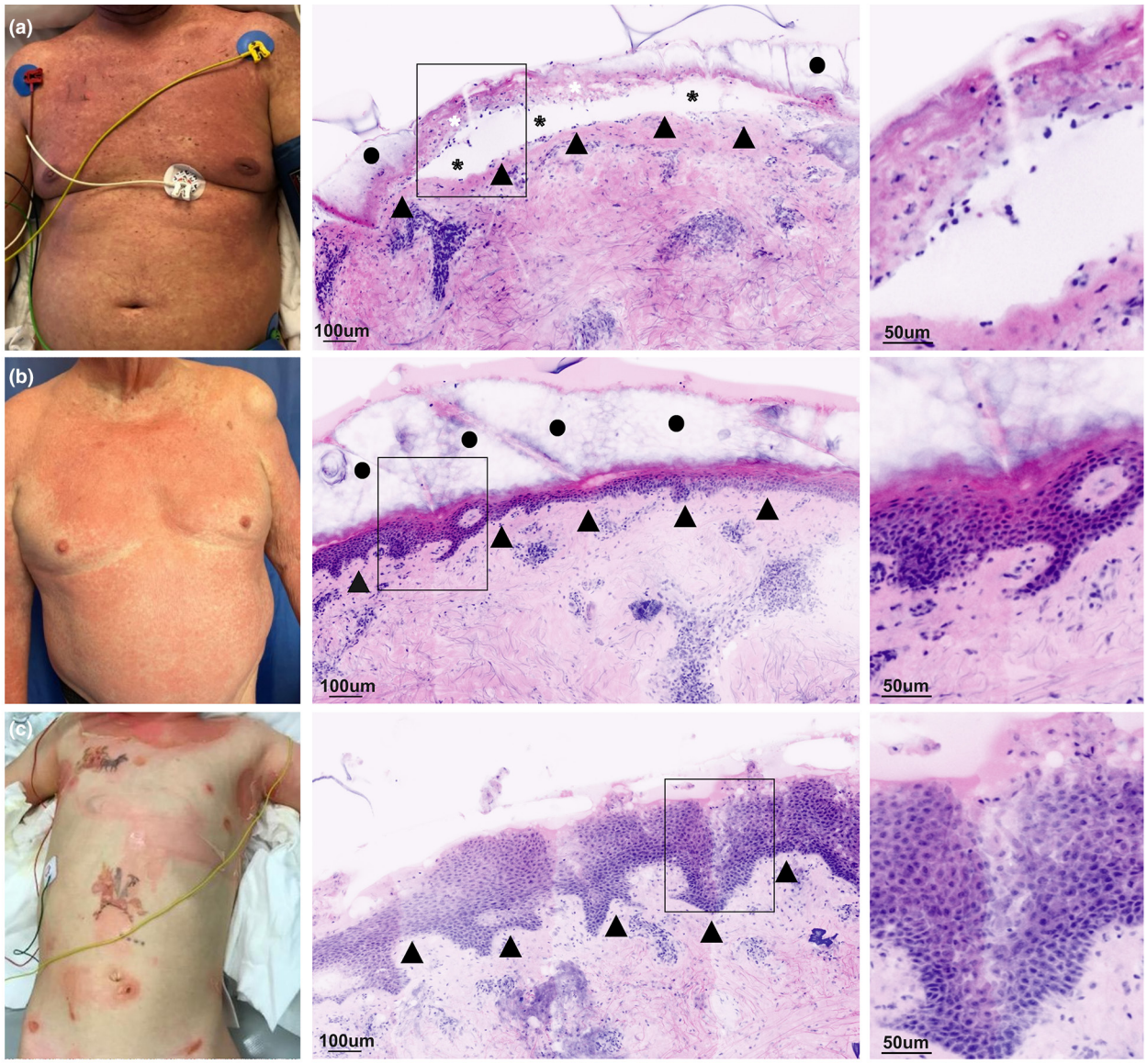


**FIGURE 1** Clinical pictures (left) and corresponding ex vivo CLSM imaging of a TEN (a), a severe maculo-papular drug eruption (b) and a staphylococcal scalded skin syndrome (c). Dermo-epidermal junction (arrowheads), stratum corneum (dots), epidermal cleavage in the basal layer (black asterisks) and confluent epidermal necrosis (white asterisks) are depicted. No epidermal cleavage in the basal layer or confluent epidermal necrosis are seen in (b) and (c). In (c), higher magnification (right) illustrates the full detachment of the stratum corneum and acantholytic keratinocytes at the surface of the epidermis. Black rectangles delineate the magnified area.

**TABLE 1** Agreement of histomorphological features found in TEN specimens examined using ex vivo CLSM and conventional histopathological analyses.

| Histological characteristics | Ex vivo CLSM ($n=8$) | H&E ($n=8$) | K | SE | Lower 95% CI | Upper 95% CI | Level of agreement |
|---|---|---|---|---|---|---|---|
| Epidermal cleavage in the basal layer | 5 (62.5%) | 6 (75%) | 0.71 | 0.27 | 0.19 | 1.24 | Substantial |
| Confluent epidermal necrosis | 5 (62.5%) | 6 (75%) | 0.71 | 0.27 | 0.19 | 1.24 | Substantial |
| Full-thickness epidermal loss | 2 (25%) | 2 (25%) | 1 | 0 | 1 | 1 | Perfect |

Abbreviations: CI, Confidence interval; H&E, haematoxylin and eosin staining; k, Fleiss' kappa; SE, Standard error.

disseminated fixed drug eruptions,[7] erythema multiforme[8] and drug-induced linear IgA dermatitis.[9,10] Further conventional histopathological analysis is thus required and, importantly, samples previously processed for ex vivo CLSM are compatible with the subsequent histopathological investigations like H&E or immunostaining processes.[6]

**TABLE 2** Diagnostic efficacy of ex vivo CLSM histomorphological features for TEN recognition.

| Histological characteristics | Sensitivity (%) | Specificity (%) | *p* |
|---|---|---|---|
| Epidermal cleavage in the basal layer | 62.5% (CI: 25.9%–89.8%) | 100% (CI: 71.7%–100%) | 0.0007 |
| Confluent epidermal necrosis | 62.5% (CI: 25.9%–89.8%) | 22.2% (CI: 3.9%–60%) | 0.048 |
| Epidermal cleavage in the basal layer or full-thickness epidermal loss | 87.5% (CI: 47%–99%) | 100% (CI: 71.7%–100%) | 0.00001 |

Abbreviation: CI, Confidence interval; *p*, *p*-value.

Therefore, it is necessary to conduct additional conventional histopathological analysis to validate or refine the definitive diagnosis. Importantly, this analysis should be compatible with the skin samples that have already been processed for ex vivo confocal laser scanning microscopy (CLSM).

## CONCLUSION

Ex vivo CLSM shows very good correlation with conventional H&E histopathology for the diagnosis of TEN, in particular for the identification of epidermal cleavage in the basal layer, essential for the distinction from SSSS. Since it does not require external technical assistance, it may provide faster, bedside diagnosis for early detection and prompt care of patients with life-threatening drug reactions.

### FUNDING INFORMATION
Open access funding provided by Universite de Lausanne.

### CONFLICT OF INTEREST STATEMENT
None to declare.

### DATA AVAILABILITY STATEMENT
The data that support the findings of this study are available from the corresponding author upon reasonable request.

### ETHICS STATEMENT
The patients in this manuscript have given written informed consent to publication of their case details.

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**How to cite this article:** Tonellotto L, Seremet T, Vernez M, Guenova E, Kuonen F. Fast, bedside diagnosis of toxic epidermal necrolysis using ex vivo confocal laser scanning microscopy: A retrospective study. J Eur Acad Dermatol Venereol. 2024;38:182–185. https://doi.org/10.1111/jdv.19424