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Effectiveness of indocyanine green in assessing tissue perfusion in lower limb open fractures: A prospective pilot study

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ABSTRACT

Background: Lower limb open fractures (LLOFs) represent complex injuries in which accurate intraoperative assessment of tissue viability is crucial to prevent infection, necrosis, and delayed healing. Clinical evaluation of perfusion is subjective and may underestimate ischemic areas, especially in high-energy trauma. Indocyanine green (ICG) fluorescence angiography provides a real-time, objective visualization of tissue perfusion and may optimize surgical decision-making during debridement.

Materials and Methods: This prospective, single-center case series included patients with Gustilo–Anderson type III open fractures and/or severe degloving injuries who underwent bone and soft-tissue reconstruction between March 2024 and July 2025. Tissue viability was first assessed clinically and then with ICG fluorescence angiography (SPY/Spy-PHI® system). Areas with < 40% relative fluorescence were considered hypoperfused. Discrepancies between clinical and ICG-defined debridement margins were measured, and postoperative outcomes were recorded.

Results: Eight patients (ten traumatic wounds) were included. In six wounds, ICG-guided assessment modified surgical debridement compared with clinical evaluation, while two showed identical margins and two were treated by clinical assessment alone. The mean ICG-defined area was 114% larger than the clinically defined area (95% CI 7.8–220.6; $p = 0.038$). Postoperative skin necrosis occurred in 3 of 10 wounds (30%): 2 of 2 managed clinically and 1 of 8 managed with ICG guidance. No ICG-related adverse events were recorded. At four weeks, 6 of 8 wounds (75%) in the ICG-guided group achieved complete healing, whereas none in the clinical group did. All soft-tissue defects healed within six months, and radiographic union occurred in 9 of 10 fractures.

Discussion: ICG fluorescence angiography enhanced intraoperative discrimination of viable from hypoperfused tissue, expanding debridement where occult ischemia was present and avoiding unnecessary excision of viable tissue. The technique improved early wound healing and reduced necrosis compared with standard clinical assessment, though the sample size limits statistical power and generalizability.

Conclusions: ICG fluorescence angiography is a safe, feasible, and objective adjunct to clinical evaluation in the management of LLOFs. Its integration into orthoplastic trauma surgery may reduce early complications and improve wound outcomes. Larger controlled studies are warranted to validate perfusion thresholds and confirm clinical benefit.

1. Introduction

Lower limb open fractures (LLOFs) are devastating injuries that involve bone disruption along with an extensive range of soft tissue damage (abrasion, crush, contusion, degloving, and loss of substance) within the injured zone. Management of lower extremity trauma is challenging and requires a multidisciplinary approach. A combined orthoplastic strategy has been shown to improve outcomes and reduce complication rates [1–3]. Early and adequate evaluation of tissue viability is of great importance in the treatment of open fractures with

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extensive soft-tissue defects, particularly in cases when the true extent of damage may not be immediately apparent [4]. Poorly perfused or necrotic tissue significantly contributes to delayed healing, increased infection risk, and surgical complications, underscoring the importance of precise intraoperative assessment. Traditionally, bone and soft tissue viability was assessed by the operating surgeon based on subjective observation, visual inspection, and palpation. These clinical measures, while widely used, are inherently prone to inaccuracies, especially in high-energy injuries and severe wounds. [5–7].

The introduction of fluorescence angiography using indocyanine green (ICG) has provided an objective method for evaluating tissue viability [8]. After intravenous administration, ICG rapidly binds plasma proteins, mainly albumin, circulates through the vascular system, and emits fluorescence under near-infrared light, which can be captured in real time using dedicated imaging systems. ICG has many favorable pharmacokinetic properties, e.g., water solubility, rapid clearance, and low toxicity, which make it a safe contrast agent [9]. .

Fluorescence imaging is one of the most popular imaging modes in several biomedical sciences for the visualization of cells and tissue [10]. It was first developed for hepatic and cardiac diagnostics, then its use was extended to oncologic surgery for sentinel lymph node mapping, in reconstructive and plastic surgery to assess flap perfusion, in vascular surgery to evaluate graft patency, and in ophthalmology for retinal angiography. In these medical fields, ICG demonstrated its value in reducing complications related to tissue ischemia.

Despite these established results and its promising function in guiding tissue viability evaluation, evidence on the use of ICG in orthopaedic surgery trauma, particularly in LLOFs, is lacking. To our knowledge, no previous studies have directly compared ICG-based tissue viability assessment with conventional clinical evaluation in this setting. This is a significant gap, as the outcome of severely traumatized tissues relies on an initial, precise debridement. Distinguishing traumatized and contaminated, yet viable tissue from non-viable can have profound consequences, both in reducing infection risk and reducing complications rate and the need for reintervention due to late-onset necrosis.

ICG fluorescence angiography may offer an objective tool to distinguish perfused from non-vital tissues, potentially reducing unnecessary excision, or, on the other hand, extending the incision more than would have been done based on the clinical appearance.

This technique allows clinicians to visualize real-time blood flow and assess tissue perfusion with minimal invasiveness. Early identification of insufficiently perfused tissue with the potential to develop ischemia or postoperative complications can guide intraoperative decision making, such as the need for flap revision, tissue resection, or a delayed procedure. [11,12].

This differentiation is crucial in the management of traumatic wounds, where rapid decisions about tissue debridement or grafting are often necessary. Although it is easy to accurately predict tissue survival in areas of high fluorescence intensity, decision-making within areas of lower intensity (*gray zones*) can be more challenging [13]. Improving the specificity of this technique will significantly increase the clinical utility and accuracy. By enhancing the clinician's ability to assess tissue viability, this approach might decrease the number of surgical debridement procedures before final reconstruction can be performed. ICG can improve outcomes by reducing unnecessary excision of viable tissue, promoting faster healing, and minimizing the risk of complications such as infection or delayed wound closure [14]. .

The present prospective pilot study had the primary aim to evaluate the feasibility, safety, and added clinical value of intraoperative ICG fluorescence angiography compared to standard clinical assessment for soft-tissue perfusion in Gustilo-Anderson type III LLOFs. Specific objectives were: (1) to quantify intraoperative discrepancies between clinically- and ICG-defined debridement margins; (2) to assess short-term postoperative outcomes including skin necrosis and wound healing at 4 weeks; and (3) to document any ICG-related adverse events. These findings aim to lay the groundwork for larger, controlled trials to validate ICG as a reliable adjunct for early, accurate identification and debridement of post-traumatic necrotic tissues.

2. Materials and methods

2.1. Study design and setting

This prospective, single-center, observational intra-patient comparative pilot study was conducted at the University Hospital of Trieste over a 17-month period (March 2024 to July 2025).

2.2. Participants

All consecutive patients presenting with Gustilo-Anderson type III B and C open fractures [3] and/or with degloving injury pattern three and four according to Arnez et al. [15] who had undergone bone and soft-tissue reconstruction after severe compound lower limb injury were considered eligible. Exclusion criteria included known allergies to iodine or indocyanine green, hyperthyroidism and major vascular injuries requiring reconstruction or repair prior to soft-tissue assessment (to avoid post-revascularization perfusion artifacts). Patients with Gustilo-Anderson type IIIC fractures (vascular injury present) were eligible only if vascular stability was achieved via temporary shunting or endovascular means before wound exposure and ICG imaging, with no ongoing ischemia at assessment time.

Within the context of consent for surgery, the operating surgeon described the procedure and the possible adverse effects of ICG. All patients provided informed consent for its intraoperative use.

2.3. Perioperative assessment and imaging protocol

Assessment occurred after standard wound exposure, hemostasis, and temporary fracture stabilization/reduction (external fixation), but strictly before any preliminary or definitive debridement, and no tourniquet was used during this phase to preserve native perfusion. Soft tissue viability was first assessed clinically (turgor, tissue color, capillary refill, temperature, bleeding on puncture) using the criteria outlined in Supplementary Table 1 to document and define preliminary debridement margins, capturing baseline traumatic soft-tissue perfusion in skin/subcutis/muscle before any excision that could artifactually improve apparent viability. ICG fluorescence angiography was then performed using SPY Elite® or SPY-PHI® systems (Stryker, Kalamazoo, MI, USA); ICG (0.1–0.3 mg/kg IV) was administered as a bolus and near-infrared (NIR) acquisition continued for 300 s, with quantitative perfusion analysis performed using SPY-Q® software. Regions with $<$ 40% relative fluorescence compared with adjacent reference tissue were a priori classified as hypoperfused [13]. Based on ICG angiography, the surgeon marked hypoperfused margins with a dermographic pen. The discrepancy between clinically and ICG-defined debridement areas was measured in centimeters. Immediate debridement, guided by the surgeon's preference (ICG or clinical and subjective knowledge), was performed and the application of negative pressure wound therapy (NPWT), following established orthoplastic protocols.

For each patient, the following data were recorded: patient demographics, fracture characteristics, clinical and ICG debridement margins, intraoperative decisions, and postoperative course and complications about wound healing, skin necrosis, infections, number of reoperations, and bone union.

2.4. Outcomes

The primary outcome was the within-wound difference between debridement areas defined clinically and those defined by ICG, expressed in cm^2 and calculated intraoperatively from the marked margins.

Secondary outcomes included early postoperative skin necrosis

within 30 days, superficial or deep surgical-site infection, the number of debridement revisions, wound status at 4 weeks (complete epithelialization yes/no), adverse reactions to ICG, and radiographic union at 6 months.

2.5. Statistical analysis

Continuous variables were summarized as mean (SD) or median (range); categorical variables as counts and percentages. Normality of paired differences between clinically and ICG-defined areas was tested with the Shapiro–Wilk test. Paired Student's *t*-test compared normally distributed paired differences; Wilcoxon signed-rank test provided a non-parametric confirmation. No formal sample-size calculation was performed due to the pilot design. Two-sided $p < 0.05$ was considered statistically significant. Analyses were conducted with IBM SPSS Statistics (version 30.0, IBM Corp., Armonk, NY, USA).

3. Results

3.1. Patients and injury profile

A total of eight patients were prospectively enrolled (7 male, 1 female; mean age 44 years, range 20–66). Across the eight patients, ten distinct soft tissue lesions were analyzed, as one patient sustained three separate traumatic wounds. Eight lesions (80%) were located on the leg (including one involving the ankle), while two lesions (20%) affected the foot. According to the Gustilo-Anderson classification, seven lesions were classified as type IIIB, and two were classified as type IIIC. In addition, two lesions were identified as degloving injuries with severity pattern 3 according to the Arnez-Tyler classification; one of these occurred concomitantly with a G-A type III B fracture.

3.2. Intraoperative assessment and primary outcome

In 6 wounds, ICG guidance altered debridement compared with the initial clinical plan; in 2 wounds, ICG and clinical margins coincided; in 2 wounds, debridement proceeded according to clinical assessment alone (Supplementary Table 2). The ICG-defined debridement area was larger than the clinically defined area in paired analysis. On average, the ICG area was 2.14 time (Fig. 1) the clinical area (95% CI 1.08–3.21).

Normality testing with the Shapiro–Wilk test proved a normal distribution of the differences ($p = 0.732$). A paired Student's *t*-test evaluated the difference between the clinical and ICG-based assessment of the area to debride ($t = 2.43$; $p = 0.038$). Furthermore, the Wilcoxon signed-rank test further showed a trend toward significance under non-parametric assumptions ($p = 0.068$).

In the 7 wounds where the ICG-guided approach demarcated a larger area than the one demarcated clinically, the mean percentage difference was 166.71% (Fig. 2).

3.3. Early postoperative outcomes

Skin necrosis occurred in 3 of 10 wounds. Necrosis developed in 2 of 2 wounds treated by clinical assessment alone and in 1 of 8 wounds treated with ICG guidance. No deep surgical-site infections were observed. No adverse reactions to ICG occurred. At 4 weeks, complete epithelialization was recorded in 6 of 8 ICG-guided wounds and in 0 of 2 clinically managed wounds (Supplementary Table 3). At 6 months, soft tissues had healed in all 8 patients, and radiographic union was documented in 9 of 10 fractures.

An illustrative case in which ICG fluorescence identified a larger hypoperfused area than clinical assessment is shown in Fig. 3, whereas Fig. 4 presents an example of ICG application in a patient with an associated degloving injury.

4. Discussion

The findings of this pilot study underscore the significant benefits of using ICG fluorescence imaging in the management of traumatic skin wounds. By providing dynamic, real-time visualization of tissue perfusion, ICG offers a more accurate and reliable assessment of tissue viability compared to traditional methods, which often rely on subjective visual inspection. This improved accuracy is crucial in cases of traumatic wounds where the extent of tissue damage is not always immediately clear, and delays in treatment or incorrect assessments can result in suboptimal healing, infection, or tissue necrosis.

ICG fluorescence angiography changed intraoperative decision-making in most wounds and revealed hypoperfused tissue not

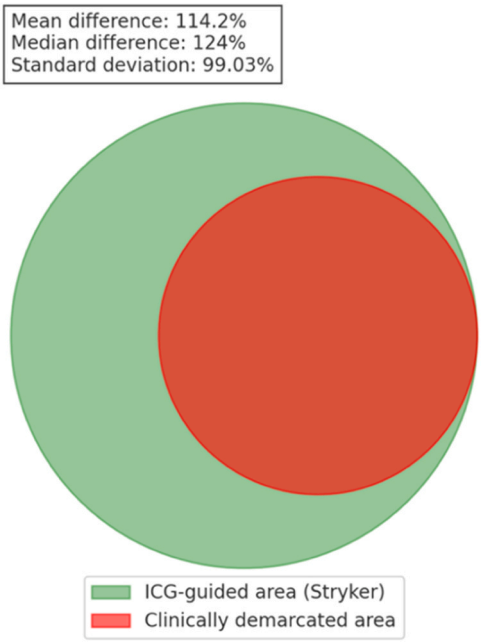

Fig. 1. Paired comparison of debridement areas: ICG-guided area (green) versus clinically demarcated area (red).

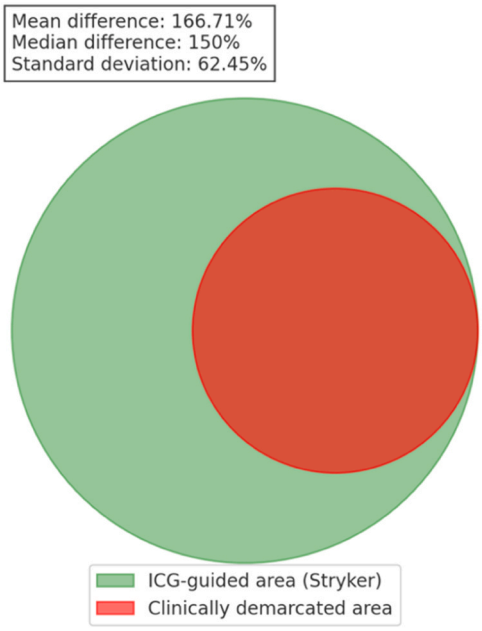

Fig. 2. Comparison of debridement margins in wounds where ICG fluorescence angiography identified a larger hypoperfused area than clinical assessment.

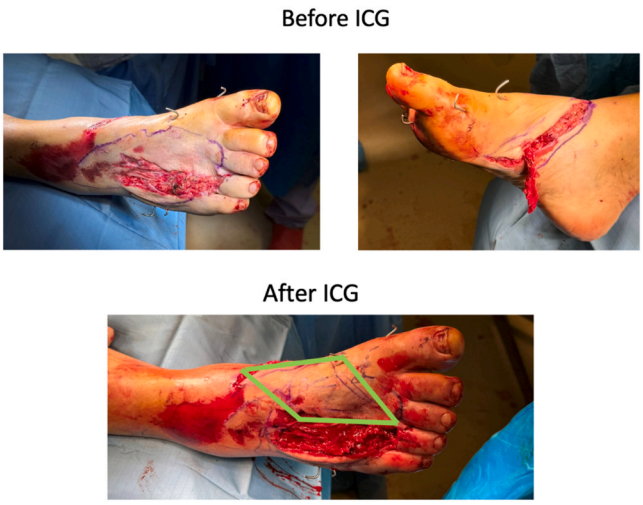

Fig. 3. Comparison of the margins of hypoperfused areas demarcated clinically (Before ICG) and using the indocyanine green (ICG) injection (After ICG).

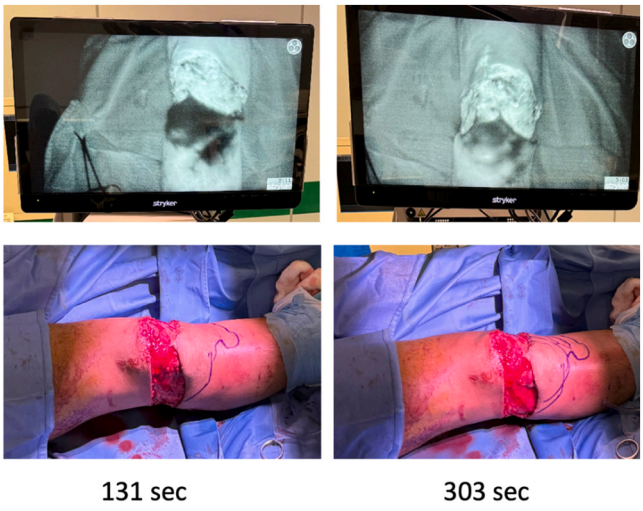

Fig. 4. Comparison of ICG perfusion in a degloving injury after two minutes and at 5 five minutes upon the administration.

detected clinically. In paired analyses, ICG-defined fields were larger than clinically planned fields, and early soft-tissue failure clustered in wounds managed without ICG guidance.

Findings of early post-operative outcome suggest that ICG-guided debridement may improve the precision of soft tissue viability assessment, reduce unnecessary excision of viable tissues and overtreatment, while also identifying non-viable tissue that still appears viable to the clinician.

ICG mostly expanded the margins of debridement beyond what clinical assessment alone had suggested. In only one case, the clinically demarcated area was bigger (25%) than the ICG-based area. This supports the concept that ICG may uncover areas of occult hypoperfusion not clear on clinical examination, while also preserving vital tissue that might seem hypoperfused.

The clinical examination for assessing tissue viability is subjective and may be inaccurate, as proven by our results, particularly in the setting of severely traumatized wounds. Our results align with reports from oncologic and reconstructive surgery, where ICG has been proven to be superior in predicting soft tissue survival and perfusion [13,14]. The significant difference in the reduced necrosis rate in the ICG group reinforces its potential role as an adjunct in the assessment of LLOFs.

Patients whose treatment was guided by ICG imaging showed improved healing outcomes compared to those assessed solely through conventional methods. Over a 4-week follow-up period, wounds in the ICG group showed faster epithelialization and reduced inflammation, with 75% achieving complete or near-complete wound closure compared to 0% in the group with clinically based debridement.

Orthoplastic trauma care can benefit from the application of ICG in the setting of LLOFs as it may reduce the number of staged procedures, allowing for an earlier and more precise excision of compromised tissue in the first place, but also leading to a reduced complication rate, such as skin necrosis, superficial and profound infection, that can delay definitive coverage and fracture healing.

ICG-fluorescence has the potential to standardize intraoperative decision-making in centers with less experience in managing complex LLOFs, offering an objective adjunct to the surgeon's experience.

This study presents several limitations that must be acknowledged. First, the small sample size and single-center design significantly limit the statistical power and generalizability of the findings. Second, the unit of analysis was the wound rather than the patient, leading to potential pseudoreplication when a single patient contributed more than one lesion. Third, the decision to follow ICG guidance or clinical judgment was left to the surgeon's discretion, introducing a risk of selection bias. Fourth, in this pilot series, we adopted a relative perfusion threshold of 40% to classify soft tissue as hypoperfused. This choice was informed by the work of Moyer and Losken [13], who used ICG angiography to predict mastectomy skin flap necrosis. In their cohort, skin with $\leq$ 25% perfusion was non-viable in approximately 90% of cases, whereas areas with $\geq$ 45% perfusion survived in 98% of cases, defining a "gray zone" between 25% and 45% of maximal perfusion in which flap outcome was uncertain. They proposed a perfusion score of around one-third of maximal perfusion as a practical cut-off to guide flap excision. We pragmatically applied a 40% cutoff to traumatized soft tissues in LLOFs based on this evidence and local expert consensus, acknowledging the need for trauma-specific validation in future studies. Within our cohort, this threshold consistently identified larger hypoperfused areas than clinical assessment alone and clustered early necrosis in wounds managed without ICG guidance, supporting its potential clinical relevance but also underscoring the necessity of larger, trauma-focused validation studies. Fifth, outcome assessment mainly focused on early complications, while other clinically relevant endpoints (e.g., time to definitive coverage, time to wound healing, length of hospitalization) were not systematically analyzed. Finally, although statistical comparison between clinical and ICG-guided debridement was performed using both parametric and non-parametric methods, results should be interpreted with caution given the small cohort size and the borderline significance observed in some tests.

Another key point is that while no adverse reactions were seen in this study, the risk of allergic reactions or iodine sensitivity in certain patients could restrict the broader use of ICG. Additionally, the need for specialized NIR imaging equipment may limit access to this technology in some clinical settings, particularly in low-resource environments. However, as NIR imaging systems become more widely available, the cost and accessibility barriers may decrease, making ICG-guided wound management more feasible across different healthcare contexts.

Another consideration is the learning curve associated with interpreting ICG fluorescence patterns. While this technology provides clear visual data, clinical expertise is still needed to accurately assess the significance of the imaging results and integrate them into surgical decision-making. Further training and standardized protocols will be necessary to ensure that ICG is used effectively in routine practice.

Furthermore, the literature holds no previous studies that have described the use of ICG in LLOFs; therefore, there is a lack of a threshold of perfusion in this field. Our center adopted the 40% perfusion threshold as a cutoff for defining tissue viability; however, this value is still somewhat arbitrary, and it was selected based on prior evidence correlating low fluorescence intensity (25–40%) with tissue necrosis in

reconstructive and vascular surgery [13,14], as well as on expert consensus within our group. Nevertheless, the transition zone between viable and non-viable tissue (grey zone) is still challenging to interpret, and necrosis may occasionally develop in areas with fluorescence above this threshold.

Future randomized trials with larger cohorts are needed to confirm the best cut-off values for the use of ICG in LLOFs and to improve the specificity of the method in borderline cases. Threshold-optimization studies should evaluate intensity cut-offs and dynamic metrics (inflow slope, time-to-peak, washout). Comparative studies with Doppler, hyperspectral imaging, or lactate measurements may improve specificity in the grey zone.

5. Conclusion

Clinical assessment of tissue perfusion following lower extremity open fractures presents a significant challenge. This prospective pilot study successfully achieved its primary aim: intraoperative ICG fluorescence angiography proved feasible and safe (no adverse events in 10 wounds), with added clinical value over clinical assessment alone in Gustilo-Anderson type III LLOFs. Specific objectives were met as follows: (1) ICG-defined debridement areas exceeded clinical margins by 114% (95% CI 7.8–20.6%, $p = 0.038$); (2) skin necrosis occurred in 1/8 ICG-guided wounds vs. 2/2 clinically managed (early healing in 6/8 ICG cases at 4 weeks); and (3) no ICG-related complications were recorded. These findings support ICG as an objective adjunct for soft-tissue perfusion assessment, laying groundwork for larger trials to validate thresholds and long-term outcomes.

Ethics

Statements and declarations

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Informed consent was obtained from all individual participants included in the study.

Patients signed informed consent regarding publishing their data.

CRediT authorship contribution statement

Giovanni Papa: Supervision, Project administration, Methodology. **Luigi Murena:** Supervision, Conceptualization. **Chiara Ratti:** Supervision, Project administration. **Eufemia Cetani:** Writing – original draft, Data curation. **Martin Iurilli:** Writing – review & editing, Writing – original draft, Investigation, Data curation, Conceptualization. **Vittorio Ramella:** Supervision, Investigation, Conceptualization. **Gianluca Canton:** Visualization, Supervision, Methodology. **Belinda Trobec:** Writing – review & editing, Visualization. **Alessio Zanata:** Writing – review & editing, Formal analysis.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.injury.2026.113259.

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