CLINICOPATHOLOGICAL CORRELATION IN ERYTHEMA INDURATUM

by

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DECLARATION

I, *Dr Lerinza van den Worm*, hereby declare that the work on which this dissertation is based is my original work (except where acknowledgements indicate otherwise) and that neither the whole work nor any part of it has been, is being, or is to be submitted for another degree in this or any other university.

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10/12/20

Signed by candidate

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Date

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List of Abbreviations

EI: Erythema Induratum

EN: Erythema Nodosum

MTB: Mycobacterium tuberculosis

TB: Tuberculosis

HIV: Human immunodeficiency syndrome

PCR: Polymerase Chain Reaction

H&E: Haematoxylin and Eosin

GMS: Grocott-Gomori's Methenamine Silver

PPD: Purified protein derivative

BCG: Bacille Calmette-Guérin

IGRA: Interferon gamma release assay

EDA: Exploratory data analysis

Chapter 1: Review of the Literature

Introduction

Erythema induratum (EI), also known as nodular vasculitis and erythema induratum of Bazin, is a chronic nodular eruption often found on the posterior aspect of the lower legs in middle aged females. [1] It is a reactive disorder to mycobacterium tuberculosis (MTB) infection although other etiological entities also exist. [2] Because MTB cannot be cultured within individual lesions the relationship between EI and MTB has been questioned. [3] Some literature suggests that the term "nodular vasculitis of Bazin" should be reserved for the portion of nodular vasculitis caused by MTB. [4]

Cutaneous TB can present with a variety of clinical manifestations but remains a rare form of extrapulmonary TB. EI is considered a type of cutaneous TB. Cutaneous tuberculosis can be divided into the route of infection: inoculated MTB from an exogenous source, from a systemic primary focus or as a tuberculid.[5] Erythema induratum is regarded as a tuberculid and therefore meant to fulfil the criteria: a positive tuberculin test, MTB involvement of the lymph nodes or internal viscera, a negative culture of the skin biopsy and resolution on antituberculosis treatment. [6] Another tuberculid has been suggested, nodular tuberculid, where changes similar to EI and papulonecrotic tuberculid occur within the lower dermis. [7]

Table 1) Types of cutaneous TB

Exogenous cutaneous TB	Tuberculosis chancre Tuberculosis verrucose cutis
Endogenous cutaneous TB	Scrofuloderma

	Orificial tuberculosis Lupus vulgaris Tuberculous gumma Acute milliary tuberculosis
Tuberculids	Papulonecrotic tuberculid Lichen scrofulosorum Phlebitic tuberculid **Erythema induratum**

The exact epidemiology of cutaneous TB is not known. A large multicenter observational study of 202998 patients with extrapulmonary TB from China gave an incidence of 0.42% of cutaneous TB. [8] Of 636 cases of extrapulmonary TB in Turkey, 1.9% had cutaneous TB. [9] Other authors have found higher incidences of cutaneous TB in all clinical cases of TB, these ranged between 2.4 and 4.8%. [10-13] A South African study reported on cutaneous conditions in patients admitted to a TB hospital, three of 105 patients had cutaneous TB, none of them were EI. [14]

Few reports on the subset who has EI, a study by Spelta et al reviewed all the cases of cutaneous TB in a state of Brazil with a high incidence of TB, over 26 years they had an incidence of only 0.44% of cutaneous TB. Of these EI was the most common presentation at 41.4% of all cutaneous TB cases. [15] Overall cutaneous TB remains a small percentage of all clinical presentations of TB.

The diagnosis of EI is more commonly based on clinical and histological findings. Although non-ulcerated lesions may heal without scarring, often subcutaneous

nodules become adherent to the skin surface and ulcerate. Lesions are usually tender but may be indolent or painful only on pressure. The course is prolonged and recurrent episodes over years are common. Individual lesions tend to involute, but new crops appear at irregular intervals. [1] Histologically it is the most common form of lobular panniculitis with vasculitis, although many controversies exist on the nature and percentage of vessel involvement.

Fig. 1a

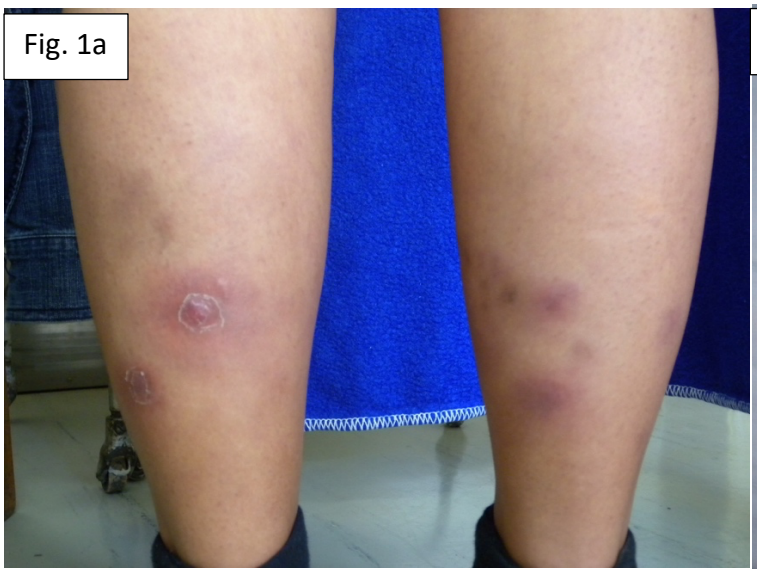
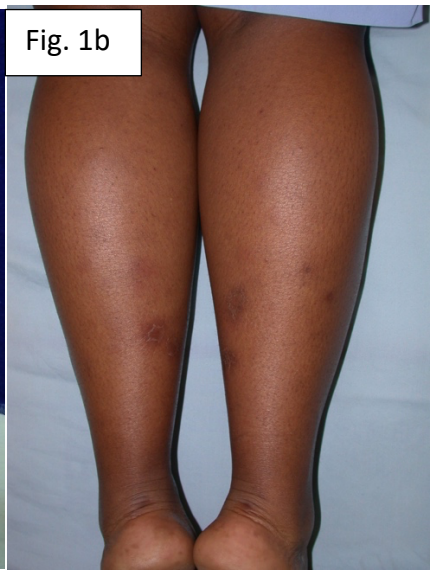


Fig. 1b



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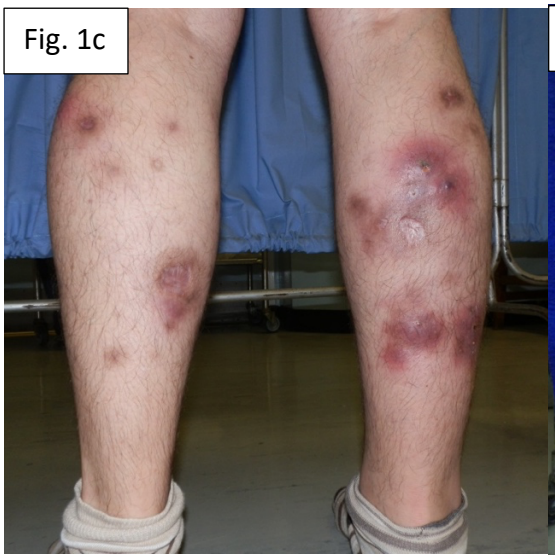
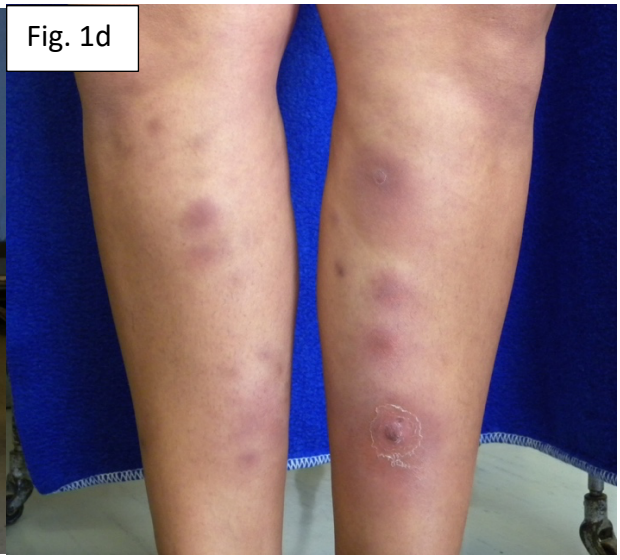


Fig. 1d



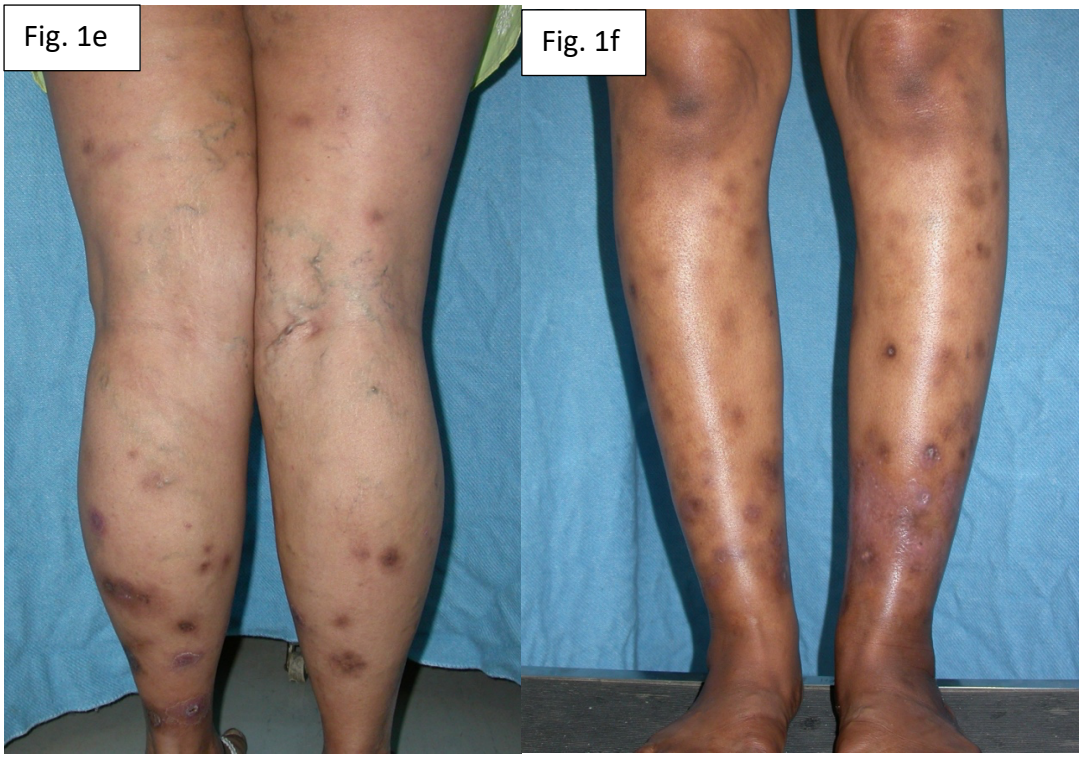


Fig 1. a-f) Clinical features of EI

Above pictures are all examples of clinicopathologically confirmed cases of EI. This illustrates the broad spectrum of clinical presentations:

Fig. 1a) Erythematous to violaceous subcutaneous nodules, note the areas of peripheral scale and adherence to the surface in the centre of the lesion, a sign of impending ulceration.

Fig. 1b) More subtle hyperpigmented to violaceous subcutaneous nodules presenting on the calves.

Fig 1c) Erythematous to violaceous nodules that ulcerate onto the surface.

Fig. 1d) Violaceous nodules predominantly on the inner calves, some showing impending central ulceration.

Fig. 1e) Violaceous subcutaneous nodules on the calves ulcerating onto the surface.

Fig 1. f) Hyperpigmentation on the upper part of the lower leg showing older healed pathology. Lower down on the shin showing ulceration.

Literature search

We conduct this literature search with the aim to peruse available literature on this rare condition, placing special emphasis on what is already available and controversies that exist.

All publications relevant to the subject were obtained via the University of Cape Town Health Sciences library. For the purposes of this study the most recent search was conducted in November 2020 using the Pubmed database and the following search terms: “erythema induratum” or “nodular vasculitis” or “erythema induratum of Bazin”. Articles were excluded if they were not relevant to the subject. Literature not published in the English language was excluded. Due to the rarity of the condition no meta-analyses, systematic reviews of placebo-controlled trials are available on the subject.

Relationship between MTB and EI

The pathogenesis of EI has been described as a type 3 hypersensitivity reaction due to the immune complex mediated vasculitis detected on histological examination. A type 4 hypersensitivity reaction has also been implicated due to the presence of T lymphocyte mediated response. MTB being the most common antigen responsible for the hypersensitivity reaction. [16] These mechanisms are clinically apparent in the

exaggerated response to purified protein derivative (PPD in the Mantoux intradermal test) in patients with erythema induratum. An interferon gamma release assay (IGRA) would also be a sensitive alternative in detecting the presence of MTB in erythema induratum. [17] Both of these investigations pose diagnostic difficulties in our setting where there will be a high false negative rate due to immunosuppressed patients with false negative Mantoux, also high incidence of Bacille Calmette-Guérin (BCG) immunisations leads to difficulties in interpretation of the Mantoux result. Our high incidence of latent tuberculosis shows a high false positive result in the IGRA. [18]

Because (MTB) cannot be cultured in the lesion the etiological relation to erythema induratum remains controversial. However, in recent years numerous studies reported the presence of MTB DNA by polymerase chain reaction (PCR). These results include positive MTB DNA detection by PCR in 77% of 65 patients, 75% of 12 patients, 14% of 65 patients and 25% of 20 patients with erythema induratum. [19-23] Although erythema induratum has been associated with other entities the association with tuberculosis remains most important in our setting where there is a high incidence of tuberculosis.

Histological features and controversies

From a histologic point of view, EI is mostly a lobular panniculitis. Initially an inflammatory infiltrate, mostly neutrophils collect in the fat lobules. There may be extensive necrosis of the adipocytes of the fat lobule and foamy histiocytes may

appear. Epithelioid histiocytes, multinucleated giant cells, and lymphocytes contribute to the granulomatous appearance of the inflammatory infiltrate in fully developed lesions. When intense vascular damage is present, extensive areas of caseous necrosis appear and the lesions show all the histopathologic attributes of tuberculosis. Caseous necrosis may extend to the overlying dermis and secondarily involve the epidermis with ulceration and discharge of liquefied necrotic fat. [1]

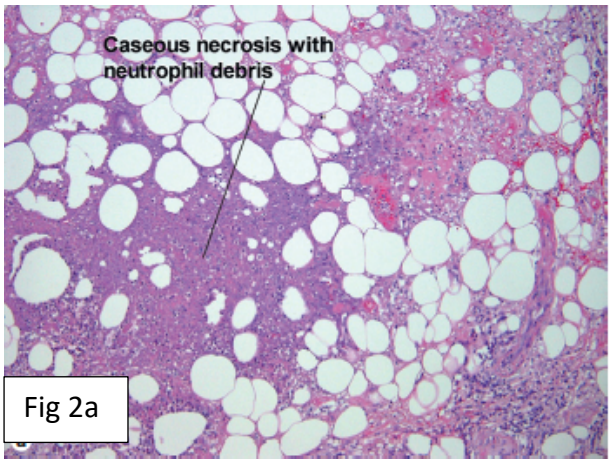


Fig 2a

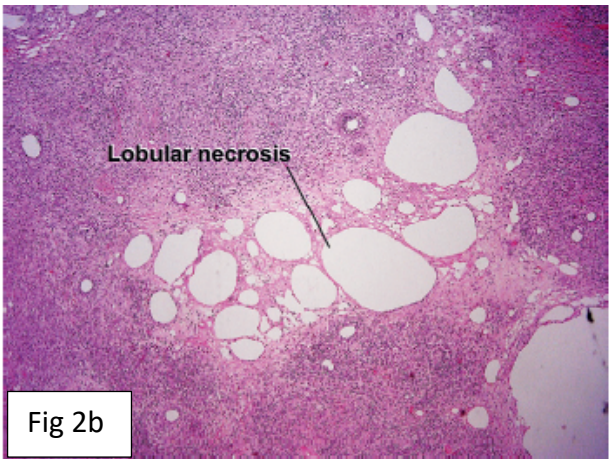


Fig 2b

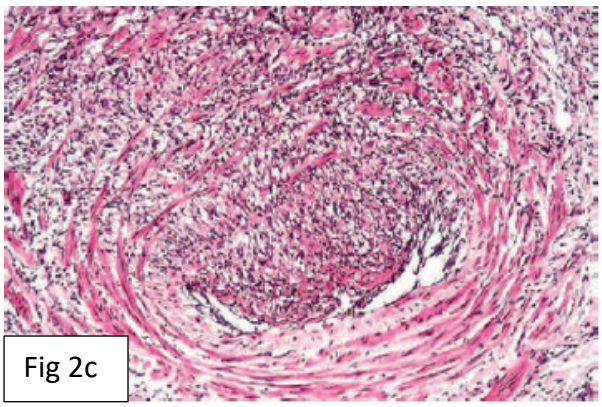


Fig 2c

Fig 2. a-c). Histological features of EI

Above pictures demonstrate a lobular panniculitis.

Fig. 2 a) Caseous necrosis with neutrophilic infiltrate, where it is evident that the fat cells are of different sizes and there is a dense inflammatory infiltrate causing caseous necrosis.

Fig. 2 b) Lobular necrosis, where it there are anucleate adipocytes of different diameter.

Fig. 2 c) High power view of inflammation of the vessel.

Controversy persists in the literature about the nature of the vessel involved. Schneider et al reviewed 19 cases where 6 had vasculitis of muscular arteries and small vessels and 6 had vasculitis of small and large vessels. [16] Segura et al demonstrated vasculitis in 90% of their 86 patients, mostly affecting small venules in the lobules, followed by venules in the septal area surrounding the fat lobules, venules in both septa and lobules and finally involvement of arteries. [24] Standard textbooks also differ in their description of the vessel involved. Rapini et al suggests vasculitis involving the arteries and small veins, [25] Weedon et al reports any size vessel being involved. [26] Elston et al suggests a neutrophilic vasculitis of the septum is often present. [27] These variations in histological features make EI a difficult and often confusing diagnosis. Whether the vasculitis is septal or lobular and the exact nature of the vessels is yet to be determined.

Granuloma formation within the histology sections seems to be a predominating feature. It is also well known that the predominate focus of inflammation withing the panniculus is lobular or septolobular. In contrast to this erythema nodosum tends to be predominately a septal panniculitis. Schneider et al divided their investigated cases into two groups. Both groups were described as septolobular panniculitides that showed varying combinations and degrees of acute and chronic inflammation, coagulative and caseation-like necrosis, and granulomatous inflammation. Poorly developed granulomas predominated, but mixed, palisading, and lipophagic granulomas also occurred. Schneider concludes that EI remains a clinicopathologic

diagnosis, but awareness of the heterogeneous histopathologic spectrum of EI will ensure a timely diagnosis and institution of antituberculous treatment. [16]

Erythema nodosum as controls

Erythema nodosum (EN) was used in our study as the comparative condition. Because we aim to let a group of dermatologists assess the features of EI blinded we included EN cases into the study. Although known as the lower leg panniculitides sharing similar clinical and histological features, EN and EI are distinctive pathologic entities. EN is the most common form of panniculitis and described as commonly occurring on the shins as opposed to EI that is described as occurring on the calves. In practice these conditions act as the main differential diagnoses for each other and are not always easy to distinguish. Therefore, a biopsy is often performed and with the added information the diagnosis becomes more apparent. As mentioned EI is predominantly a lobular panniculitis, whereas in EN the focus of inflammation occurs primarily within the septum of the panniculus. Where MTB is often the triggering factor for EI, EN on the other hand has many identified potential triggers including infectious agents, medications and underlying systemic disorders. Rarely MTB can be a triggering factor in EN, but more often streptococcal throat infection or gastrointestinal infections are the causative organisms. [28]

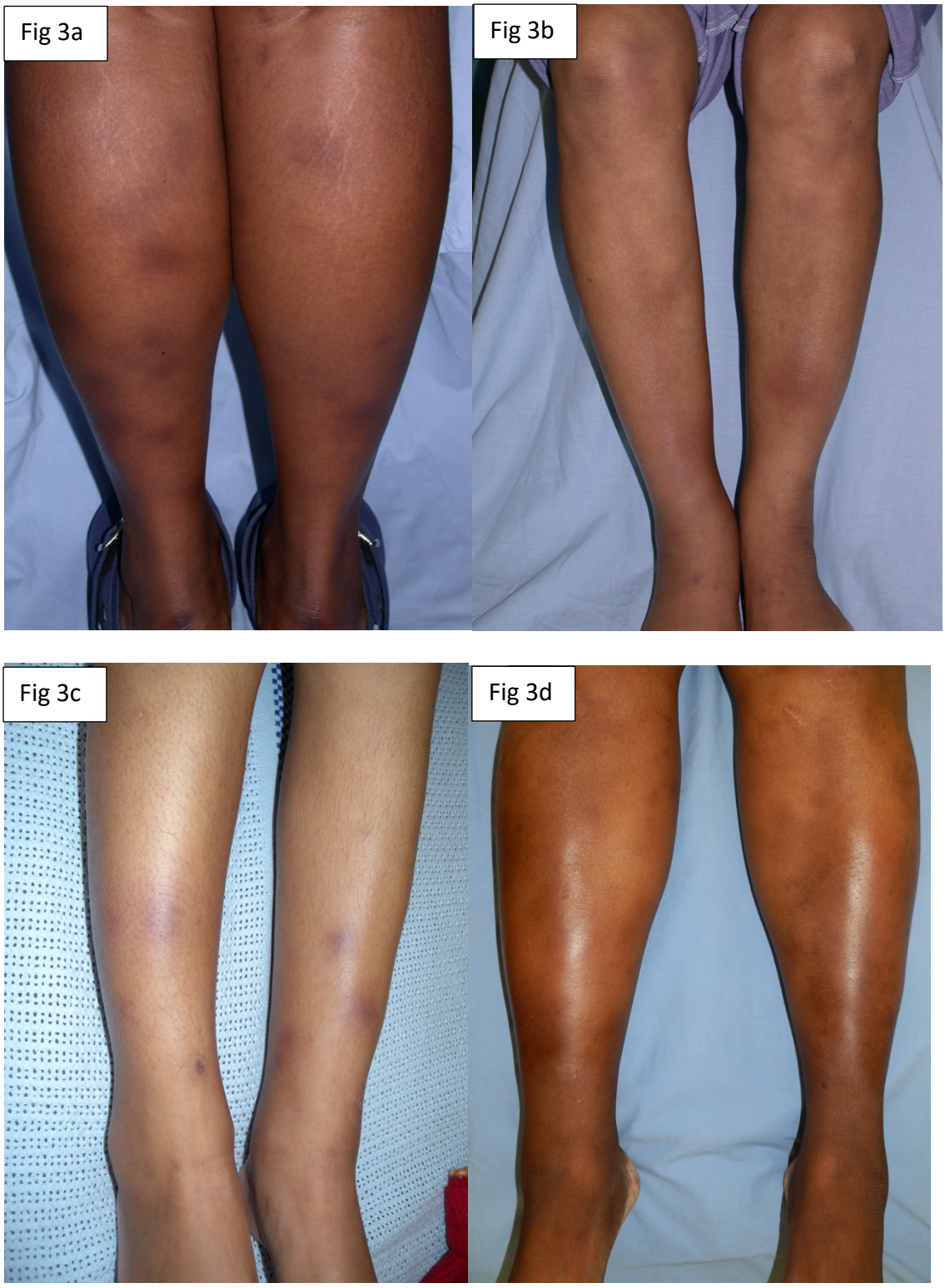


Fig. 3 a-d) Clinical features of EN

Above four different patients with clinicopathologically confirmed EN can be witnessed.

Fig. 3 a) Erythematous to hyperpigmented subcutaneous nodules can be seen on the calves.

Fig. 3 b) Erythematous nodule over the shin

Fig. 3 c) Violaceous nodules predominantly on the shins

Fig. 3 d) Hyperpigmented induration of older lesions, note the absence of ulceration.

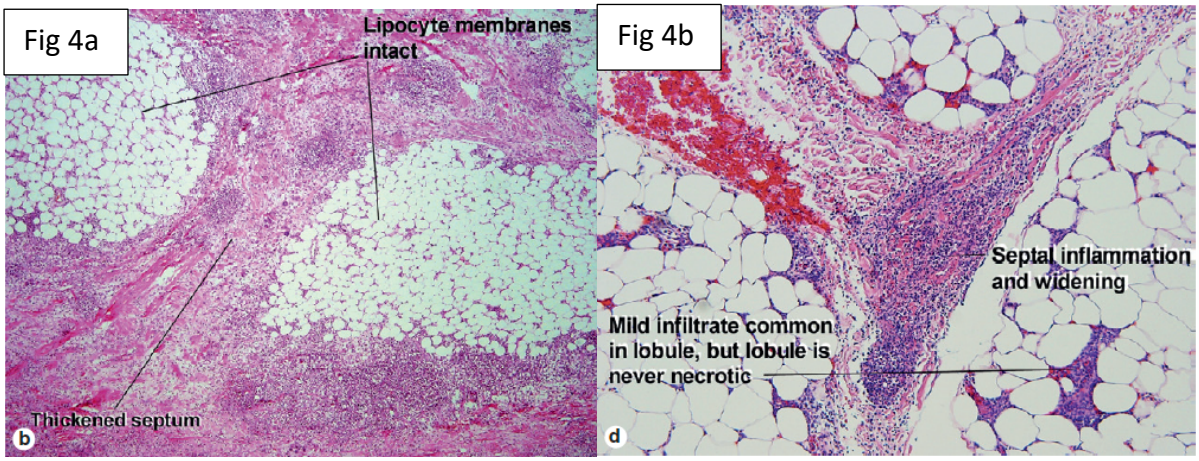


Fig. 4 a-b) Histological features of EN

Fig. 4 a) A septal panniculitis where the focus of inflammation is predominantly within the septum causing widening of the septum.

Fig. 4 b) Although there might be inflammation within the lobular component, the focus of inflammation remains within the septum. Granulomas might be found within the septum.

A deep biopsy to classify the type of panniculitis is often performed. Although trephine punches have been proposed for biopsies of panniculitides, an incisional biopsy with a larger scalpel that extends through the subcutaneous fat is preferred. [29] This might lead to unfavorable wound healing especially if the section is taken from the lower leg. It is for this reason that some clinicians aim to not take a biopsy

for cases of EN, believing that there are no harmful underlying conditions to exclude and that the condition is generally self-limiting. It is therefore important to study the clinical features of EN and EI and see if these entities can be distinguished based only on clinical examination.

Gaps in the literature

The literature available on erythema induratum is lacking. Small cohorts are available due to the rarity of the disease and the exact incidence is not known. A previous study done in South Korea identified 53 patients prospectively and in Brazil 54 cases retrospectively. [30, 31] In Spain 86 cases were collected retrospectively at 2 institutions and 65 cases at one institution over an undefined period of time. [20, 22, 24] These make out the largest cohorts. Numerous smaller studies and case reports also report on erythema induratum. Few studies correlate the clinical and histological features of this condition.

A study done by Rademaker et al, reviewed clinical and histologic features of 26 cases of EI. The characteristic cutaneous lesions on the legs of middle-aged women were violaceous, indurated nodules, 1 to 2 cm in diameter, which were painful, occasionally ulcerated, and could heal with scarring. The histologic features were varied but were predominantly those of a vasculitis with a septolobular and lobular panniculitis. [32] These cases were confirmed by having a strongly positive Mantoux test. This is not feasible in our setting where there is a high incidence of latent TB and a high incidence of HIV, making the interpretation of the result difficult.

Previous South African studies [16, 23] focused predominantly on the histological features and did not correlate them with the clinical features in their description. Considering the high prevalence of TB in South Africa more local studies are needed to describe this cutaneous manifestation of MTB. This is particularly important when considering the diagnosis of TB since EI can be the presenting feature of underlying TB where there is strong enough immunity to suppress other clinical symptoms of TB for example marked chest X-ray changes and systemic symptoms.

Once the diagnosis of EI is confirmed a systemic source of TB should be investigated. There is no clear mention in the literature of what investigations should be performed nor whether EI is associated with pulmonary or extrapulmonary TB. Patients who develop hypersensitivity reactions in EI generally have good cellular immunity, and the source of TB infection can be difficult to detect. Few case reports mention extrapulmonary TB related to the clinical diagnosis of EI. Examples include hepatic TB, renal tuberculosis and endometrial TB. [33-35]

These case reports are likely an overrepresentation and pulmonary TB is underrepresented because it is the most common presentation of TB. There is however mention that childhood cutaneous TB represents 18% to 82% of all cutaneous tuberculosis cases. Scrofuloderma and lupus vulgaris (both cutaneous presentations of TB) are the two most common clinical forms in children. An increase in the number of tuberculids, especially lichen scrofulosorum, has been observed in the last several years.[36]

There is little mention of specific management of EI in the literature. As for the management of EN an underlying precipitant should be treated, in the case of EI this is most often systemic antituberculosis therapy to treat MTB infection. Thereafter non-steroidal anti-inflammatories plus rest and elevation of the legs constitute the mainstay of treatment. For resistant cases colchicine and potassium iodide are instituted.[28] A single case report mentions topical isoniazid as specific treatment for EI. [37]

After extensive review of the literature it is evident that EI is a rare presentation of a hypersensitivity reaction to TB that is under reported in the literature. Available research highlights the difficulties in making an accurate diagnosis. There is conflicting evidence for the clinical and histological features of EI. Also Mantoux and IGRA testing pose interpretation difficulties in our South African setting.

Although these clinical and histological guidelines are given in most texts, practically it remains a difficult clinical and histological diagnosis. EI is also a rare condition making large prospective studies with conclusive results difficult. Using EN as an alternative clinical condition we will test a group of dermatologists on their ability to distinguish EI and EN. This will be based first on the clinical images and then the histological features will be revealed. The ability of the initial assessment and the contributing value of the histological features will be assessed. We aim to investigate what clinical and histological features contribute most to an accurate diagnosis of EI.

References:

1. Requena L, Sánchez Yus E. Panniculitis. Part II. Mostly lobular panniculitis. Journal of the American Academy of Dermatology. 2001;45(3):325-61; quiz 62-4.
2. Wolf KT, G. . Fitzpatrick dermatology in general medicine. 3rd ed. New York: McGraw-Hill; 1987.
3. Mascaró JM, Jr., Baselga E. Erythema induratum of bazin. Dermatologic clinics. 2008;26(4):439-45, v.
4. Cho KH, Lee DY, Kim CW. Erythema induratum of Bazin. International journal of dermatology. 1996;35(11):802-8.
5. Khadka P, Koirala S, Thapaliya J. Cutaneous Tuberculosis: Clinicopathologic Arrays and Diagnostic Challenges. Dermatology research and practice. 2018;2018:7201973.
6. Kuramoto Y, Aiba S, Tagami H. Erythema induratum of Bazin as a type of tuberculid. Journal of the American Academy of Dermatology. 1990;22(4):612-6.
7. Jordaan HF, Schneider JW, Abdulla EA. Nodular tuberculid: a report of four patients. Pediatric dermatology. 2000;17(3):183-8.
8. Kang W, Yu J, Du J, Yang S, Chen H, Liu J, et al. The epidemiology of extrapulmonary tuberculosis in China: A large-scale multi-center observational study. PloS one. 2020;15(8):e0237753.
9. Ilgazli A, Boyaci H, Basyigit I, Yildiz F. Extrapulmonary tuberculosis: clinical and epidemiologic spectrum of 636 cases. Archives of medical research. 2004;35(5):435-41.
10. Varshney A, Goyal T. Incidence of various clinico-morphological variants of cutaneous tuberculosis and HIV concurrence: a study from the Indian subcontinent. Annals of Saudi medicine. 2011;31(2):134-9.
11. Kivanç-Altunay I, Baysal Z, Ekmekçi TR, Köslü A. Incidence of cutaneous tuberculosis in patients with organ tuberculosis. International journal of dermatology. 2003;42(3):197-200.
12. Yates VM, Ormerod LP. Cutaneous tuberculosis in Blackburn district (U.K.): a 15-year prospective series, 1981-95. The British journal of dermatology. 1997;136(4):483-9.
13. Kumar B, Rai R, Kaur I, Sahoo B, Muralidhar S, Radotra BD. Childhood cutaneous tuberculosis: a study over 25 years from northern India. International journal of dermatology. 2001;40(1):26-32.
14. McLachlan I, Visser WI, Jordaan HF. Skin conditions in a South African tuberculosis hospital: Prevalence, description, and possible associations. International journal of dermatology. 2016;55(11):1234-41.
15. Spelta K, Diniz LM. cutaneous tuberculosis: a 26-year retrospective study in an endemic area of tuberculosis, vitória, espírito santo, brazil. Revista do Instituto de Medicina Tropical de Sao Paulo. 2016;58:49.
16. Schneider JW, Jordaan HF. The histopathologic spectrum of erythema induratum of Bazin. The American Journal of dermatopathology. 1997;19(4):323-33.
17. Angus J, Roberts C, Kulkarni K, Leach I, Murphy R. Usefulness of the QuantiFERON test in the confirmation of latent tuberculosis in association with erythema induratum. The British journal of dermatology. 2007;157(6):1293-4.

18. Vera-Kellet C, Peters L, Elwood K, Dutz JP. Usefulness of Interferon-γ release assays in the diagnosis of erythema induratum. Archives of dermatology. 2011;147(8):949-52.
19. James WB, TG. Elston, EM. Andrew's Diseases of the skin. 11th ed: Elsevier; 2011.
20. Baselga E, Margall N, Barnadas MA, Coll P, de Moragas JM. Detection of Mycobacterium tuberculosis DNA in lobular granulomatous panniculitis (erythema induratum-nodular vasculitis). Archives of dermatology. 1997;133(4):457-62.
21. Chen YH, Yan JJ, Chao SC, Lee JY. Erythema induratum: a clinicopathologic and polymerase chain reaction study. Journal of the Formosan Medical Association = Taiwan yi zhi. 2001;100(4):244-9.
22. Vieites B, Suárez-Peñaranda JM, Pérez Del Molino ML, Vázquez-Veiga H, Pardo F, Del Rio E, et al. Recovery of Mycobacterium tuberculosis DNA in biopsies of erythema induratum--results in a series of patients using an improved polymerase chain reaction technique. The British journal of dermatology. 2005;152(6):1394-6.
23. Schneider JW, Jordaan HF, Geiger DH, Victor T, Van Helden PD, Rossouw DJ. Erythema induratum of Bazin. A clinicopathological study of 20 cases and detection of Mycobacterium tuberculosis DNA in skin lesions by polymerase chain reaction. The American Journal of dermatopathology. 1995;17(4):350-6.
24. Segura S, Pujol RM, Trindade F, Requena L. Vasculitis in erythema induratum of Bazin: a histopathologic study of 101 biopsy specimens from 86 patients. Journal of the American Academy of Dermatology. 2008;59(5):839-51.
25. Rapini R. Practical dermatopathology. Second ed. USA: Elsevier; 2012.
26. Patterson JW. Weedon's skin pathology. 5th ed: Elsivier; 2020 6th March 2020.
27. Elston DF, T. Ko, C. . Dermatopathology. 3rd ed: Elsevier; 2019 17th August 2018.
28. Gilchrist H, Patterson JW. Erythema nodosum and erythema induratum (nodular vasculitis): diagnosis and management. Dermatologic therapy. 2010;23(4):320-7.
29. Tok J, Abrahams I, Ravits MA, Silvers DN. Surgical pearl: the trephine punch for diagnosing panniculitis. Journal of the American Academy of Dermatology. 1996;35(6):980-1.
30. Magalhães TS, Dammert VG, Samorano LP, Litvoc MN, Nico MMS. Erythema induratum of Bazin: Epidemiological, clinical and laboratorial profile of 54 patients. The Journal of dermatology. 2018;45(5):628-9.
31. Cho KH, Kim YG, Yang SG, Lee DY, Chung JH. Inflammatory nodules of the lower legs: a clinical and histological analysis of 134 cases in Korea. The Journal of dermatology. 1997;24(8):522-9.
32. Rademaker M, Lowe DG, Munro DD. Erythema induratum (Bazin's disease). Journal of the American Academy of Dermatology. 1989;21(4 Pt 1):740-5.
33. Daher Ede F, Silva Júnior GB, Pinheiro HC, Oliveira TR, Vilar Mdo L, Alcântara KJ. Erythema induratum of Bazin and renal tuberculosis: report of an association. Revista do Instituto de Medicina Tropical de Sao Paulo. 2004;46(5):295-8.
34. Ribeiro R, Patrício C, Pais da Silva F, Silva PE. Erythema induratum of Bazin and Ponçet's arthropathy as epiphenomena of hepatic tuberculosis. BMJ case reports. 2016;2016.

35. De Silva HJ, Goonetilleke AK, De Silva NR, Amarasekera LR, Jayawickrama US. Erythema induratum (of Bazin) in a patient with endometrial tuberculosis. Postgraduate medical journal. 1988;64(749):242-4.
36. Sethuraman G, Ramesh V. Cutaneous tuberculosis in children. Pediatric dermatology. 2013;30(1):7-16.
37. Mei X, Zhao J. Successful treatment of erythema induratum with topical application of antituberculous drugs: A case report. Medicine. 2017;96(49):e9010.

Chapter 2: Manuscript

Title Page

Clinicopathological correlation in Erythema Induratum

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Running head: Clinicopathological correlation erythema induratum

Abstract

Background - Erythema induratum (EI) is a reactive disorder to mycobacterium tuberculosis infection, a diagnosis not to be missed. Erythema nodosum (EN) is the main clinical differential of EI, but a distinctly different pathological condition that can be difficult to distinguish from EI.

Methods – In this retrospective review we assess clinical and histological features of 40 EI cases and 16 EN cases. Six experienced dermatologists blindly diagnosed these cases based on clinical images, thereafter the histology was revealed, and they adjusted their diagnoses accordingly. Fleiss Kappa statistics were applied to determine inter-rater variability. A multi-variate logistic regression model determined the clinical and histological features that contribute most to an accurate diagnosis.

Results - After assessing the clinical picture 48.8% of the EI cases and 74% of the EN cases were correctly diagnosed. With added histology results 67.1% EI and 81.2% EN cases were correct. EI cases showed inter-rater variability of 0.478 (p-value < 0.01) before and 0.469 (p-value < 0.01) after histology was revealed. These features combined in a logistic regression model had a higher diagnostic accuracy than the assessors with regard to EI cases. The model was accurate in 100% and 80% of EI and EN cases respectively.

Conclusions - While the study was limited by its retrospective nature and small sample size, valuable features (ulceration, vasculitis and lobular or septal panniculitis) were identified. A biopsy of the lower leg markedly increased the diagnostic accuracy, but there was less concordance between assessors, more research is needed to confirm these results.

Publication ready manuscript:

Introduction

Erythema induratum (EI), also known as nodular vasculitis and erythema induratum of Bazin, is a chronic nodular eruption often found on the posterior aspect of the lower legs in middle aged females. [1] It is a reactive disorder to mycobacterium tuberculosis (MTB) infection although other etiological entities also exist. [2] EI is regarded as a tuberculid and therefore should fulfil the criteria: a positive tuberculin test, mycobacterium tuberculosis involvement of the lymph nodes or internal viscera, a negative culture of the skin biopsy and resolution on antituberculosis treatment. [3] Because MTB cannot be cultured within individual lesions the relationship between EI and MTB has been questioned. [4] Some literature suggests that the term “nodular vasculitis of Bazin” should be reserved for the portion of nodular vasculitis caused by MTB. [5]

Clinically EI presents as subcutaneous nodules on the calves. Although non-ulcerated lesions may heal without scarring, often subcutaneous nodules become adherent to the skin surface and ulcerate. Lesions are usually tender but may be indolent or painful only on pressure. The course is prolonged and recurrent episodes over years are common. Individual lesions tend to involute, but new crops appear at irregular intervals. [1] Histologically it is the most common form of lobular panniculitis with vasculitis, although many controversies exist on the nature of the vessel involved and percentage who has vessel involvement.

The pathogenesis of EI has been described as a type 3 hypersensitivity reaction due to the immune complex mediated vasculitis detected on histological examination. A type 4 hypersensitivity reaction has also been implicated due to the presence of T lymphocyte mediated response. MTB being the most common antigen responsible for the hypersensitivity reaction. [6] These mechanisms are clinically apparent in the exaggerated response to purified protein derivative (PPD in the Mantoux intradermal test) in patients with EI. An interferon gamma release assay would also be a sensitive alternative in detecting the presence of MTB in EI. [7] Both of these investigations pose diagnostic difficulties in the South African setting where there will be a high false negative rate due to immunosuppressed patients with false negative Mantoux, also high incidence of bacille Calmette-Guérin (BCG) immunisations leads to difficulties in interpretation of the Mantoux result. Our high incidence of latent tuberculosis shows a high false positive result in the interferon-gamma release assay. [8]

From a histologic point of view, EI is mostly a lobular panniculitis. Initially an inflammatory infiltrate, mostly neutrophils collect in the fat lobules. There may be extensive necrosis of the adipocytes of the fat lobule and foamy histiocytes may appear. Epithelioid histiocytes, multinucleated giant cells, and lymphocytes contribute to the granulomatous appearance of the inflammatory infiltrate in fully developed lesions. When intense vascular damage is present, extensive areas of caseous necrosis appear and the lesions show all the histopathologic attributes of tuberculosis. Caseous necrosis may extend to the overlying dermis and secondarily involve the epidermis with ulceration and discharge of liquefied necrotic fat. [1]

Controversy persists in the literature about the nature of the vessel involved. Schneider et al reviewed 19 cases where 6 had vasculitis of muscular arteries and small vessels and 6 had vasculitis of small and large vessels. [6] Segura et al demonstrated vasculitis in 90% of their 86 patients, mostly affecting small venules in the lobules, followed by venules in the septal area surrounding the fat lobules, venules in both septa and lobules and finally involvement of arteries. [9] Standard textbooks also differ in their description of the vessel involved. Rapini et al suggests vasculitis involving arteries and small veins, [10] Weedon et al reports any size vessel being involved. [11] Elston et al suggests a neutrophilic vasculitis of the septum is often present.[12] These variations in histological features make EI a difficult and often confusing diagnosis. Whether the vasculitis is septal or lobular and the exact nature of the vessels is yet to be determined.

Granuloma formation within the histology sections seems to be a predominating feature. It is also well known that the predominant focus of inflammation withing the panniculus is lobular or septolobular. In contrast to this EN tends to be predominantly a septal panniculitis. Schneider et al divided their investigated cases into two groups. Both groups were described as septolobular panniculitides that showed varying combinations and degrees of acute and chronic inflammation, coagulative and caseation-like necrosis, and granulomatous inflammation. Poorly developed granulomas predominated, but mixed, palisading, and lipophagic granulomas also occurred. Schneider concludes that EI remains a clinicopathologic diagnosis, but awareness of the heterogeneous histopathologic spectrum of EI will ensure a timely diagnosis and institution of antituberculous treatment.[6]

Erythema nodosum (EN) was used in our study as the comparative condition. Because we aimed to let a group of dermatologists assess the features of EI blinded, we included EN cases in the study. Although known as the lower leg panniculitides sharing similar clinical and histological features, EN and EI are distinctive pathologic entities. EN is the most common form of panniculitis and described as commonly occurring on the shins as opposed to EI that is described as occurring on the calves. In practice these conditions act as the main differential diagnoses for each other and are not always easy to distinguish. Therefore, a biopsy is often performed and with the added information the diagnosis becomes more apparent. As mentioned EI is predominantly a lobular panniculitis, whereas in EN the focus of inflammation occurs primarily within the septum of the panniculus. Where MTB is often the triggering factor for EI, EN on the other hand has many identified potential triggers including infectious agents, medications and underlying systemic disorders. Rarely MTB can be a triggering factor in EN, but more often streptococcal throat infection or gastrointestinal infections are the causative organisms. [13]

A deep biopsy to classify the type of panniculitis is often performed. Although trephine punches have been proposed for biopsies of panniculitides, an incisional biopsy with a larger scalpel that extends through the subcutaneous fat is preferred. [14] This might lead to unfavorable wound healing especially if the section is taken from the lower leg. It is for this reason that some clinicians aim to not take a biopsy for cases of EN, believing that there are no harmful underlying conditions to exclude and that the condition is generally self-limiting. Therefore, we aim to study the clinical features of EN and EI and see if these entities can be distinguished based only on clinical examination and what contributing value a biopsy adds.

Materials and Methods

We describe 40 cases of EI and 16 cases of EN as controls. Cases were collected retrospectively at a single institution (Groote Schuur Hospital, Cape Town, South Africa) over a period of 14 years 2004-2018. The cases were identified via a database used by the department of dermatology, containing all cases seen in their clinic where biopsies were performed. These cases are all discussed at an interdepartmental dermatology-pathology meeting where a final diagnosis is reached. This study was approved by the Human Research Ethics Committee of the University of Cape Town. Consent was taken from each patient before the commencement of the biopsy.

The inclusion criteria were:

1) Cases where a clinical-pathological diagnosis of EI or EN was made.
2) Patients above the age of 12.

The exclusion criteria were:

1) Outstanding clinical records, images or histology reports.
2) Cases with insufficient or poor clinical images.

Clinical data collected included demographics, description of lesions and a brief presenting complaint with relevant clinical history. All biopsy specimens underwent histopathological assessment that included hematoxylin-eosin (H&E) and various special stains (Ziehl–Neelsen, Brown-Brenn, Grocott-Gomori's Methenamine Silver).

Most cases also had specimens submitted for microbiologic examination including culturing methods to detect mycobacterium tuberculosis.

Six qualified dermatologists with collectively 108 years of clinical experience in dermatology took part in the assessment. The EI and EN cases where randomized and presented in a Microsoft PowerPoint presentation. Each case had 2 components:

1) Relevant clinical history and images.
2) The corresponding microscopic description in the histology report.

For each case, after viewing the first component, the assessor recorded their likely diagnosis (EI/EN/unsure). The second component then followed where they had another opportunity to record their diagnosis (EI/EN/unsure).

Diagnostic accuracy was studied using confusion matrices created using the diagnosis based on clinical impression alone. The results were then compared to the diagnostic accuracy with the additional microscopic description. Inter-rater variability was assessed using Fleiss'-kappa. Demographic, clinical, laboratory data and assessors' answers were stored in Microsoft Excel. Data analysis was performed in R version 4.0.2 and Python version 3.7.4.

Exploratory data analysis (EDA) was performed on the full dataset to determine any possible relationships that might aid in the successful diagnosis of EI. Feature Importance analysis was performed using Scikit-learn's Random Forest algorithm to determine the features with the greatest predictive power. Input features for

consideration were the location of the lesion, ulceration, vasculitis and septal or lobular panniculitis. The best features were then selected for multivariate logistic regression. Incomplete data was removed from the modeling process, this removed seven entries from the original dataset. The dataset was further split into a training and testing test. The testing set consisted of 33% of the remaining data and was used to compare the model diagnostic accuracy to that of the assessors.

Results

In this study, 56 patients with lower leg panniculitis were included, 40 (71%) EI cases and 16 (29%) EN cases to serve as controls. Of the EI cases 38 (95%) were female with an average age of 33.3 years (+/- 11.8 years). The EN cases consisted of 15 (94%) female patients and an average age of 41 years (+/- 9.7 years).

Clinically detectable ulceration was detected in 25 (63%) of the EI cases and 1 (6%) of the EN cases. The predominant distribution of the lesions in EI was on both the calves and shins of the lower legs 22 (55%), followed by 11 (28%) distributed on the calves, 3 (8%) spread across the thighs and lower legs, 3 (8%) on the shins and 1 (3%) widespread on the legs and arms. The EN predominant distribution of lesions included both the calves and the shins 5 (31%), thighs and lower legs 4 (25%), predominantly shins 3 (19%), legs and arms 3 (19%) and 1 (6%) on the calves (Table 1).

Tissue cultures aimed specifically to detect mycobacterium tuberculosis (MTB) were performed in 19 of the EI cases and 11 of the EN cases, all cultures were negative.

Special stains performed included Ziehl-Neelsen, Brown-Brenn and Grocott-Gomori's Methenamine Silver; they were all negative. The predominant focus of infiltration in the panniculus (septal, lobular or septolobular) was reported in 36/40 of the EI cases and 16/16 of the EN cases. The EI cases were predominantly lobular 24/36 (67%) or septolobular 10/36 (28%), two of the cases were described as having a septal predominance. Of the EN cases there was a clear predominance of a septal pattern 14/16 (88%), with two cases described as septolobular. Granulomas were detected in 37 (95%) of the reported 39 EI cases and 10 (71%) of the reported 14 EN cases. Both necrotizing and non-necrotizing granulomas were described in EI and EN cases. Vasculitis was present in 23/37 (62%) of the EI cases, three cases did not report on the presence or absence of vasculitis. No vasculitis was detected in 12 of the EN specimens, four did not report on vasculitis. (Table 2).

Six assessors reviewed the 56 EI and EN cases in a randomized presentation. After assessing the clinical images together with the presenting complaint and relevant history 48.8% of the EI cases and 74% of the EN cases were correctly diagnosed. After each clinical image the microscopic description in the histology report was revealed, with this added information the assessors now correctly identified 67.1% of the EI cases and 81.2% of the EN cases. At times the assessors were unsure of the diagnosis, this was the case in 12.1% EI and 12.5% EN clinical diagnoses and 18.3% EI and 12.5% EN diagnoses with added microscopic findings. Overall inter-rater variability showed fair agreement when considering the clinical images only with a Fleiss'-Kappa score of 0.321 (p-value < 0.01). Inter-rater variability was improved to 0.424 (p-value < 0.01) after the addition of the microscopic findings, now showing moderate inter-rater variability. EI cases showed inter-rater variability of

0.478 (p-value < 0.01) before and 0.469 (p-value < 0.01) after histology was revealed. EN cases had inter-rater variability of 0.331 (p-value < 0.01) before and 0.549 (p-value < 0.01) after histology was revealed. (Table 3)

The final logistic regression model disregarded the location of the lesion and included ulceration, vasculitis and lobular or septal panniculitis. The model showed an in-sample accuracy of 96.6% compared to an out-of-sample accuracy of 94.1%. On the test set, the assessors were accurate in 79.2% and 83.3% of cases for EI and EN respectively. The model was accurate in 100% and 80% of EI and EN cases respectively, showing an improvement in the diagnosis of EI and similar results to the assessors in the case of EN.

Discussion

Our cohort of 40 EI patients is similar in size to the largest cohorts in the literature. A previous study done in South Korea identified 53 patients prospectively and in Brazil 54 cases retrospectively. [15-16]. In Spain 86 cases were collected retrospectively at 2 institutions and 65 cases at one institution. [9, 17, 19] These make out the largest cohorts, few studies correlate the clinical and histological features of this condition.

There was a clear predominance of female cases, this is in keeping with previous reported literature. EI lesions were distributed predominantly on the anterior and posterior legs, contradicting the usual interpretation that EI presents on the calves and EN on the shins. [13]. Ulceration, a helpful clinical feature, was present in only

62% of EI cases but notably absent in the EN cases. Although a previous large cohort of 54 patients detected an ulceration rate of only 24% of EI cases. [15]

Microbiological cultures and special stains for infective organisms on histology were all negative. Because MTB cannot be cultured in the lesion the etiological relation to EI remains controversial. However, in recent years numerous studies reported the presence of MTB DNA by polymerase chain reaction (PCR). These results include positive MTB DNA detection by PCR in 77% of 65 patients, 75% of 12 patients, 14% of 65 patients and 25% of 20 patients with EI. [17-21]

Histological features of EI were predominantly septal or septolobular. Vasculitis was present in 62% of cases, previous literature reports 90%. [9] A similar study done by Rademaker et al, reviews clinical and histologic features of 26 cases of EI. The characteristic cutaneous lesions on the legs of middle-aged women were violaceous, indurated nodules, occasionally ulcerated, and could heal with scarring. The histologic features were varied but were predominantly those of a vasculitis with a septolobular and lobular panniculitis. [22]

For the assessors, diagnostic accuracy increased from 48.8% to 67.1% for EI and from 74% to 81.2% for EN after the histology was revealed. We saw a slight decrease in inter-rater reliability in the diagnosis of EI compared with a large increase in inter-rater reliability for the diagnosis of EN. Thus, for the EI cases the addition of histological features did not lead to more concordance between assessors. Assessors had substantial agreement on the histological features of EN.

Feature importance analysis showed that the location of the lesion was not as important as ulceration, vasculitis and septal or lobular panniculitis. The single most important feature being a septal panniculitis in EN in 88% of cases. (Fig. 1)

Although EI has been associated with other entities the association with tuberculosis, a diagnosis not to be missed, remain most important in our setting where there is a high incidence of tuberculosis. While the study was limited by its retrospective nature and small sample size, valuable features (ulceration, vasculitis and lobular or septal panniculitis) were identified. These features combined in a logistic regression model had a higher diagnostic accuracy than the assessors with regards to EI cases. A biopsy of the lower leg markedly increased the diagnostic accuracy, but did not lead to increased concordance between assessors, more research is needed to confirm these results.

Acknowledgements

There was no source of external funding.

References

1. Requena L, Sánchez Yus E. Panniculitis. Part II. Mostly lobular panniculitis. Journal of the American Academy of Dermatology. 2001;45(3):325-61; quiz 62-4.

2. Wolf KT, G. . Fitzpatrick dermatology in general medicine. 3rd ed. New York: McGraw-Hill; 1987.

3. Kuramoto Y, Aiba S, Tagami H. Erythema induratum of Bazin as a type of tuberculid. Journal of the American Academy of Dermatology. 1990;22(4):612-6.

4. Mascaró JM, Jr., Baselga E. Erythema induratum of bazin. Dermatologic clinics. 2008;26(4):439-45, v.

5. Cho KH, Lee DY, Kim CW. Erythema induratum of Bazin. International journal of dermatology. 1996;35(11):802-8.

6. Schneider JW, Jordaan HF. The histopathologic spectrum of erythema induratum of Bazin. The American Journal of dermatopathology. 1997;19(4):323-33.

7. Angus J, Roberts C, Kulkarni K, Leach I, Murphy R. Usefulness of the QuantiFERON test in the confirmation of latent tuberculosis in association with erythema induratum. The British journal of dermatology. 2007;157(6):1293-4.

8. Vera-Kellet C, Peters L, Elwood K, Dutz JP. Usefulness of Interferon-γ release assays in the diagnosis of erythema induratum. Archives of dermatology. 2011;147(8):949-52.

9. Segura S, Pujol RM, Trindade F, Requena L. Vasculitis in erythema induratum of Bazin: a histopathologic study of 101 biopsy specimens from 86 patients. Journal of the American Academy of Dermatology. 2008;59(5):839-51.

10. Rapini R. Practical dermatopathology. Second ed. USA: Elsevier; 2012.

11. Patterson JW. Weedon's skin pathology. 5th ed: Elsivier; 2020 6th March 2020.

12. Elston DF, T. Ko, C. . Dermatopathology. 3rd ed: Elsevier; 2019 17th August 2018.

13. Gilchrist H, Patterson JW. Erythema nodosum and erythema induratum (nodular vasculitis): diagnosis and management. Dermatologic therapy. 2010;23(4):320-7.

14. Tok J, Abrahams I, Ravits MA, Silvers DN. Surgical pearl: the trephine punch for diagnosing panniculitis. Journal of the American Academy of Dermatology. 1996;35(6):980-1.

15. Magalhães TS, Dammert VG, Samorano LP, Litvoc MN, Nico MMS. Erythema induratum of Bazin: Epidemiological, clinical and laboratorial profile of 54 patients. The Journal of dermatology. 2018;45(5):628-9.

16. Cho KH, Kim YG, Yang SG, Lee DY, Chung JH. Inflammatory nodules of the lower legs: a clinical and histological analysis of 134 cases in Korea. The Journal of dermatology. 1997;24(8):522-9.

17. Baselga E, Margall N, Barnadas MA, Coll P, de Moragas JM. Detection of Mycobacterium tuberculosis DNA in lobular granulomatous panniculitis (erythema induratum-nodular vasculitis). Archives of dermatology. 1997;133(4):457-62.

18. Chen YH, Yan JJ, Chao SC, Lee JY. Erythema induratum: a clinicopathologic and polymerase chain reaction study. Journal of the Formosan Medical Association = Taiwan yi zhi. 2001;100(4):244-9.

19. Vieites B, Suárez-Peñaranda JM, Pérez Del Molino ML, Vázquez-Veiga H, Pardo F, Del Rio E, et al. Recovery of Mycobacterium tuberculosis DNA in biopsies of erythema induratum--results in a series of patients using an improved polymerase chain reaction technique. The British journal of dermatology. 2005;152(6):1394-6.

20. James WB, TG. Elston, EM. Andrew's Diseases of the skin. 11th ed: Elsevier; 2011.

21. Schneider JW, Jordaan HF, Geiger DH, Victor T, Van Helden PD, Rossouw DJ. Erythema induratum of Bazin. A clinicopathological study of 20 cases and detection of Mycobacterium tuberculosis DNA in skin lesions by polymerase chain reaction. The American Journal of dermatopathology. 1995;17(4):350-6.

22. Rademaker M, Lowe DG, Munro DD. Erythema induratum (Bazin's disease). Journal of the American Academy of Dermatology. 1989;21(4 Pt 1):740-5.

Tables for publication

Table 1. Patient demographics and lesion distribution

	EI	EN
	n (%) or mean and SD	n (%) or mean and SD
Female gender	38 (95%)	15 (94%)
Age	33.3 +/- 11.8	41 +/- 9.7
Ulceration of lesion	25(63%)	1 (6%)
Predominant lesion distribution:		
Shins	3 (8%)	3 (19%)
Calves	11 (28%)	1 (6%)
Anterior and posterior lower legs	22 (55%)	5 (31%)
Thighs and lower legs	3 (8%)	4 (25%)
Legs and arms	1 (3%)	3 (19%)

SD = standard deviation; EI = Erythema induratum; EN = Erythema nodosum.

Table 2. Histopathological and Microbiological properties

	EI	**EN**
	n (%)	n (%)
Positive culture (positive/total)	0/19 (0%)	0/11 (0%)
Special histology stains: (positive/total)		
Ziehl-Neelsen	0/37 (0%)	0/11 (0%)
Brown-Brenn	0/10 (0%)	0/3 (0%)
GMS	0/33 (0%)	0/8 (0%)
Distribution of infiltrate: (n/total)		
Lobular	24/36 (67%)	0/16 (0%)
Septal	2/36 (6%)	14/16 (88%)
Septolobular	10/36 (28%)	2/16 (12%)
Granulomas (n/total)		
Present	37/39 (95%)	10/14 (71%)
Absent	2/39 (5%)	4/14 (29%)
Vasculitis (n/total)		
Present	23/37 (62%)	0/12 (0%)
Absent	14/37 (38%)	12/12 (100%)

SD = standard deviation; EI = Erythema induratum; EN = Erythema nodosum; GMS = Grocott-Gomori's Methenamine Silver

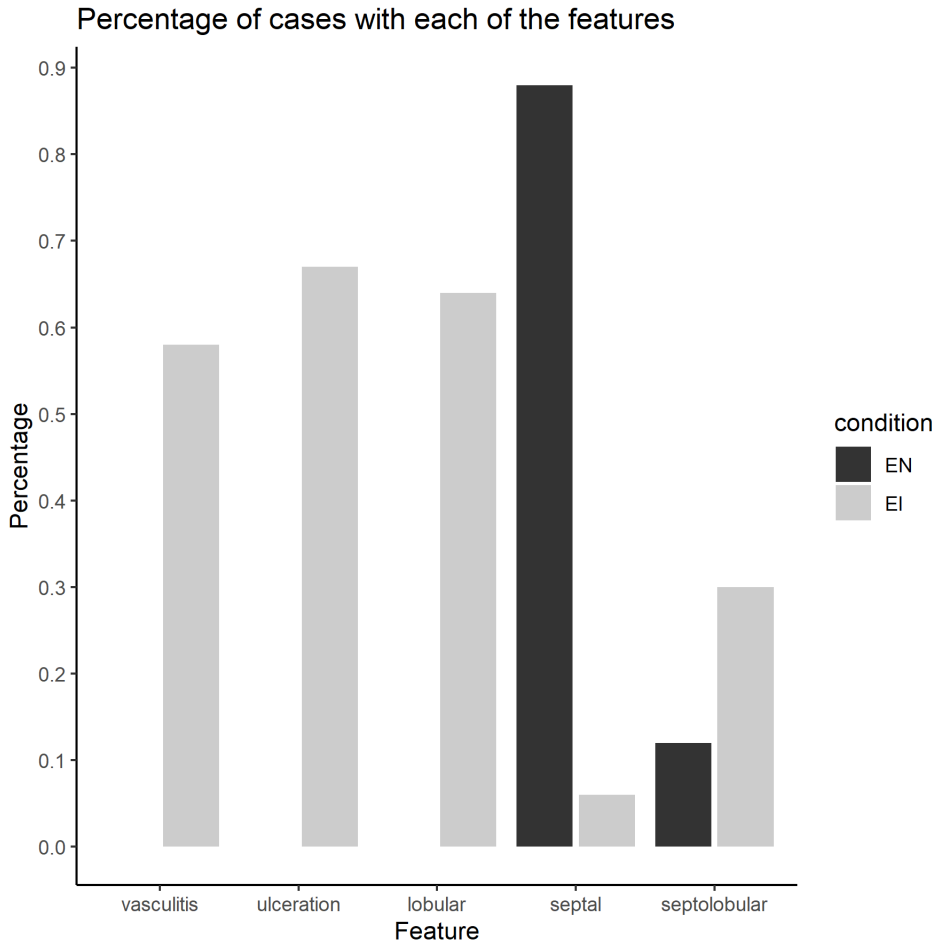
Table 3. Assessor diagnosis from clinical presentation alone and combined with histology report

	Actual EI % (n)	Actual EN % (n)
Diagnosis on clinical presentation		
Diagnosed EI	48.8% (117)	13.5% (13)
Diagnosed EN	39.2% (94)	74% (71)
Diagnosed Unsure	12.1% (29)	12.5% (12)
Diagnosis on clinical and histological presentation		
Diagnosed EI	67.1% (161)	6.2% (6)
Diagnosed EN	14.6% (35)	81.2% (78)
Diagnosed Unsure	18.3% (44)	12.5% (12)

EI = Erythema induratum; EN = Erythema nodosum

Figures for publication

Figure 1. Percentage of EI and EN cases containing each of the features.



EI = Erythema induratum; EN = Erythema nodosum

Chapter 3 Appendices:

Appendix 1 – Additional tables not for publication

Table 4. Fleiss' Kappa inter-rater reliability scores.

		EI	EN	UNSURE
KAPPA	**Clinical**	0.478	0.331	-0.050
	Added histology	0.469	0.549	0.143
P VALUE	**Clinical**	<0.01	<0.01	0.147
	Added histology	<0.01	<0.01	<0.01

EI = Erythema induratum; EN = Erythema nodosum

Table 5. Multivariate logistic regression model and Assessor diagnostic accuracy on test data.

	EI	EN	Entire Sample
Multivariate Logistic Regression Model	100%	80%	94.1%
Assessors	79.2%	83.3%	80.4%

EI = Erythema induratum; EN = Erythema nodosum

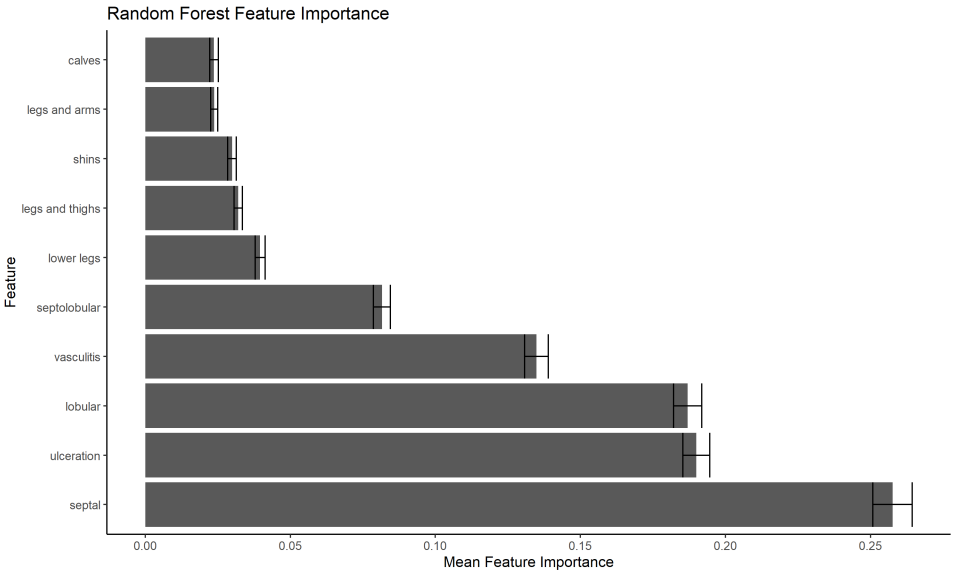
Interpretation of Kappa values

K	**Interpretation**
< 0	Poor agreement
0.01 – 0.20	Slight agreement
0.21 – 0.40	Fair agreement
0.41 – 0.60	Moderate agreement
0.61 – 0.80	Substantial agreement
0.81 – 1.00	Almost perfect agreement

Referenced from: 1. Landis JR, Koch GG. The measurement of observer agreement for categorical data. Biometrics. 1977;33(1):159-74.

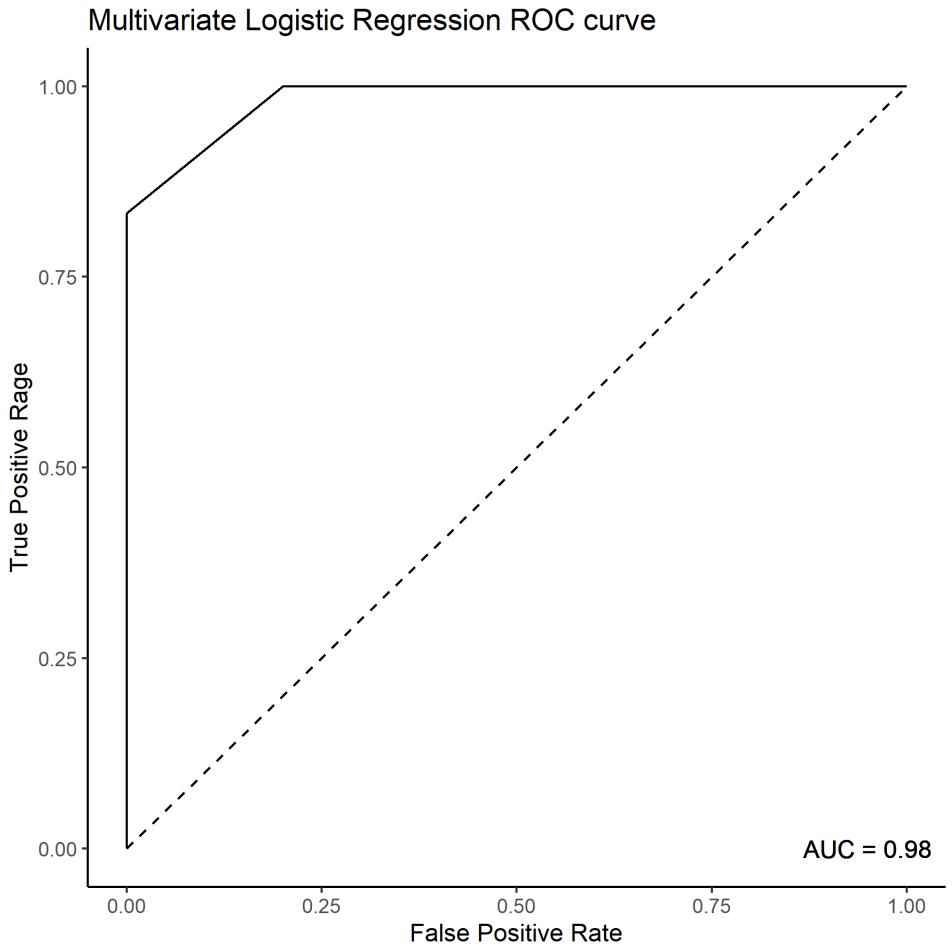
Appendix 2 – Additional figures not for publication

Figure 2. Random forest feature importance.



The feature importance of the different features can be seen in Figure 2. The bars show the mean feature importance over all the trees in the random forest model. The error bars are one standard deviation above and below the mean.

Figure 3. Multivariate Logistic Regression ROC curve.



The test set, not used during the training process, was used to compare the out of sample performance of the multivariate logistic regression model with the average diagnosis of the assessors. Figure 3 shows the ROC curve of the out of sample results with an AUC of 0.98 showing that the model performs significantly better than random.

Appendix 3 – HREC Approval letter

UNIVERSITY OF CAPE TOWN
Faculty of Health Sciences
Human Research Ethics Committee

Room E53-46 Old Main Building
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Website: www.health.uct.ac.za/fhs/research/humanethics/forms

15 June 2018

HREC REF: 361/2018

Dr RM Ngwanya
Dermatology
Ward G23, NGSH

Dear Dr Ngwanya

PROJECT TITLE: CLINICAL-HISTOLOGICAL CORRELATION IN ERYTHEMA INDURATUM (MMED Candidate - Dr L van den Worm)

Thank you for submitting your study to the Faculty of Health Sciences Human Research Ethics Committee.

It is a pleasure to inform you that the HREC has **formally approved** the above-mentioned study.

Approval is granted for one year until the 30 June 2019.

Please submit a progress form, using the standardised Annual Report Form if the study continues beyond the approval period. Please submit a Standard Closure form if the study is completed within the approval period.
(Forms can be found on our website: www.health.uct.ac.za/fhs/research/humanethics/forms)

Please quote the HREC REF in all your correspondence.

Please note that the ongoing ethical conduct of the study remains the responsibility of the principal investigator.

Please note that for all studies approved by the HREC, the principal investigator **must** obtain appropriate institutional approval, where necessary, before the research may occur.

The HREC acknowledge that the student, Dr Lerinza Van Den Worm will also be involved in this study.

Yours sincerely

PROFESSOR M BLOCKMAN
CHAIRPERSON, FHS HUMAN RESEARCH ETHICS COMMITTEE
Federal Wide Assurance Number: FWA00001637.
Institutional Review Board (IRB) number: IRB00001938
This serves to confirm that the University of Cape Town Human Research Ethics Committee complies to the Ethics Standards for Clinical Research with a new drug in patients, based on the Medical

Research Council (MRC-SA), Food and Drug Administration (FDA-USA), International Convention on Harmonisation Good Clinical Practice (ICH GCP), South African Good Clinical Practice Guidelines (DoH 2006), based on the Association of the British Pharmaceutical Industry Guidelines (ABPI),and Declaration of Helsinki (2013) guidelines.
The Human Research Ethics Committee granting this approval is in compliance with the ICH Harmonised Tripartite Guidelines E6: Note for Guidance on Good Clinical Practice (CPMP/ICH/135/95) and FDA Code Federal Regulation Part 50, 56 and 312.

HREC 361/2018

Appendix 4 – STROBE Checklist

STROBE Statement—Checklist of items that should be included in reports of ***cohort studies***

	Item No	Recommendation	Comments
Title and abstract	1✓	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	p. 28
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	p. 28
Introduction			
Background/rationale	2✓	Explain the scientific background and rationale for the investigation being reported	p. 29-32
Objectives	3✓	State specific objectives, including any prespecified hypotheses	p. 29-32
Methods			
Study design	4✓	Present key elements of study design early in the paper	p. 33-35
Setting	5✓	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	p. 33-35
Participants	6✓	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	p. 33-35
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed	p. 33-35
Variables	7✓	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	p. 33-35
Data sources/ measurement	8✓	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group:	p. 33-35
Bias	9✓	Describe any efforts to address potential sources of bias:	p. 33-35
Study size	10✓	Explain how the study size was arrived at: *Prior studies were examined – this further discussed in the manuscript*	In discussion
Quantitative variables	11✓	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why.	p. 33-35
Statistical methods	12✓	(*a*) Describe all statistical methods, including those used to control for confounding	p. 33-35
		(*b*) Describe any methods used to examine subgroups and interactions	p. 33-35
		(*c*) Explain how missing data were addressed	p. 33-35
		(*d*) If applicable, explain how loss to follow-up was addressed	N/A

		(*e*) Describe any sensitivity analyses	p. 33-35
Results			
Participants	13✓	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	p. 35-37
		(b) Give reasons for non-participation at each stage	p. 35-37
		(c) Consider use of a flow diagram	N/A
Descriptive data	14✓	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	p. 35-37
		(b) Indicate number of participants with missing data for each variable of interest	p.35-37
		(c) Summarise follow-up time (eg, average and total amount)	N/A
Outcome data	15✓	Report numbers of outcome events or summary measures over time	p.35-37
Main results	16✓	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	N/A
		(*b*) Report category boundaries when continuous variables were categorized	N/A
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	N/A
Other analyses	17✓	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	p.35-37
Discussion			
Key results	18✓	Summarise key results with reference to study objectives	p. 37
Limitations	19✓	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	p. 39
Interpretation	20 ✓	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	p. 39
Generalisability	21 ✓	Discuss the generalisability (external validity) of the study results	p. 39
Other information			

Funding	22✓	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	p. 39

*Give information separately for exposed and unexposed groups.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at http://www.strobe-statement.org.

Appendix 5 – Instruction for authors, International Journal of Dermatology

Author Guidelines

IJD SUBMISSION FORMS

Online Submission
Contact the Editorial Office

1. ABOUT *IJD*

Published monthly, the *International Journal of Dermatology* (*IJD*) is specifically designed to provide dermatologists around the world with a regular, up-to-date source of information on all aspects of the diagnosis and management of skin diseases. Accepted articles regularly cover clinical trials, education, morphology, pharmacology and therapeutics, case reports, and reviews. Additional features include tropical medicine reports, news, correspondence, and proceedings and transactions.

IJD is guided by a distinguished, international editorial board and emphasizes a global approach to continuing medical education for physicians and other providers of health care with a specific interest in problems relating to the skin.

2. MANUSCRIPT CATEGORIES

IJD invites the following types of submission:

Case Report*
A report of 400–600 words, illustrated by no more than three illustrations. This category offers a means for rapid communication about a single subject.

Clinical Trial
An article of 700–1200 words concerning a drug evaluation. This category provides rapid publications and is meant to be a succinct presentation with a minimum of graphs and tables.

Commentary*
An editorial 700–1200 words in length with approximately five references. The author may express his or her opinion without complete documentation.

Clinicopathological Challenge*
A photographic essay that includes both a clinical and a pathological photograph in color. The diagnosis and legends for the photographs should be listed after the references in the article. The article should be no more than 2 pages in length and contain 4-5 references.

Correspondence*
Letters to the editor and short notes. Contributions should not exceed 600 words, two figures, and 5 references. In order to offer rapid dissemination of accepted manuscripts, Correspondence items will be published online-only. Online-only correspondence items are assigned to an issue of the journal, but are excluded from the print edition. Online-only correspondence items are e-paginated and are fully citable and indexable.

Dermatological Surgery
An article relating to the surgical aspects of treatment. Article types may include Review, Report or Correspondence format.

Education
An article about the methodology of curriculum and instruction in dermatology, about 2500 words.

On a Human Scale* (by invitation only)
An article that relates to social, economic, cultural, artistic and humanitarian aspects of medicine. The length of the article should not exceed 1200 words including a short summary of the topic addressed. A brief author biography and photo should be submitted with the article. If you have a topic that you feel would fit nicely in this section, please send a note to **IntJDerm@gmail.com** for approval to submit.

Pharmacology and Therapeutics
An article relating to the treatment of diseases and to the pharmacology of dermatologically-

related drugs. (Can include Clinical Trials, Reviews, Reports, and Correspondence. The latter is preferred for reports of adverse drug reactions.) When referring to a drug, please use the generic name approved by the United States Food and Drug Administration or recognized as the United States Adopted Name. At the end of the manuscript, please list the American Trade names.

Reminiscence
An article on the history of dermatology or skin diseases; also a biographic account of an historic or noteworthy figure in dermatology.

Report
An original article including, whenever possible, an Introduction, Materials and Methods or Case Report(s), Results, Comment, and References. A Structured Abstract of not more than 250 words must be included and should consist of four paragraphs labeled Background, Methods, Results, and Conclusions. Also, it should describe the problem studied, how the study was performed, the main results, and what the author(s) concluded from the results. The article should range from 2500-3000 words.

Review
A major didactic article that clarifies and summarizes the existing knowledge in a particular field. It should not be an exhaustive review of the literature, and references should not exceed 50 in number. Tables, diagrams, and selected figures are often helpful and preferred. The length is left to the judgment of the author, although it generally should not exceed 5000 words. Topics may include updates in clinically relevant basic science and cutaneous biology. A list of 10 multiple choice and/or true and false questions should be listed at the end of the article to provide additional educational challenge to the reader. An abstract is required, though it need not be structured.

Tropical Medicine Rounds
An article dealing with the diseases and special problems encountered by dermatologists working in the tropics. Article submissions should follow the Report, Case Report, or Correspondence format.

Updates in Medicine
This contribution to the journal should be 700–1200 words in length with sufficient references to document important points. It is not essential that the contribution be heavily referenced as it is meant to serve as an update for dermatologists in various fields of medicine and is not portrayed to be an extensive or exhaustive review of the literature. However, it would be very helpful if pertinent and salient references are included, not only for documentation purposes, but also for additional reading.

Medical Genetics
Report, Review or Correspondence format should be followed.

*No abstract required

3. SUBMISSION OF MANUSCRIPTS

Submissions should be made online at the *IJD* **ScholarOne Manuscripts site** (formerly known as Manuscript Central). New users should first create an account (do not upload document files at this time). Once a user is logged onto the site, submissions should be made via the Author Center.

Revised manuscripts must be submitted as revisions as directed by the ScholarOne website. Do not resubmit a revision as a new manuscript as this may result in re-review and considerable delay. The revision should be complete and contain all the tables and figures. Do not resubmit the original manuscript with your revision.

Submission of a manuscript will be held to imply that it contains original unpublished work and is not being submitted for publication elsewhere at the same time. The author must supply a full statement to the Editor about all submissions and previous reports that might be regarded as redundant or duplicate publication of the same or very similar work.

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4. PREPARATION OF MANUSCRIPTS

Manuscripts must be written in English and must comply with these instructions in every detail.

Text should be supplied in a word processed format such as Microsoft Word for Windows. Charts and tables are considered textual and should be supplied in the same format. Figures (illustrations, diagrams, photographs) should be supplied in gif, jpeg, tif or eps format.

All manuscripts must be typed in 12 pt font with lines double spaced and margins of at least 2.5 cm.

Abbreviations must be defined when first used, both in the abstract and in the main text.

Manuscripts must be as succinct as possible. Text must comply with the word and figure limits defined in Section 2. If authors consider that a manuscript should not conform to the limits specified, exceptionally good reasons must be clearly provided in a cover letter accompanying the submission. Repetition of information or data in different sections of the manuscript must be carefully avoided.

Manuscripts should, where appropriate, include:

Title Page

The first page of all manuscripts should contain the following information:
1) the title of the paper
2) surnames (family names),initials of each author, and their degree (if any)
3) name of the institution(s) at which the research was conducted
4) name, address, telephone number and email address of corresponding author
5) manuscript word count (excluding abstract and references), table and figure count
7) any conflict of interest disclosures (see Section 5)
8) a running head not exceeding 50 characters

Abstracts

Authors submitting Reports should note that structured abstracts (maximum 250 words) are required. The structured abstract should adopt the format: Background, Methods, Results, Conclusions.

Review articles require abstracts (maximum 250 words) but they need not be structured.

Abstracts should not contain citations.

Text

This should in general, but not necessarily, be divided into sections with the headings: Abstract, Introduction, Materials and Methods, Results, Discussion, Acknowledgements, References, Tables, Table and figure legends. Figures should be submitted as separate files. The acknowledgements should include a statement of all funding sources that supported the work.

Please submit the full text of the manuscript, including the abstract, references, tables and legends as a single document. The title page may be included as page 1 of the main manuscript document or can be uploaded as a separate file, but must be included.

Tables and Figures

Tables should not be inserted in the appropriate place in the text but should be included at the end of the manuscript, each on a separate page.

Figures (illustrations, diagrams, photographs) should be supplied in gif, jpeg, tif or eps format and submitted as separate electronic files.

Tables and figures should be referred to in text as follows: Fig. 1, Figs. 2–4; Table 1, Tables 2 and 3. The place at which a table or figure is to be inserted in the printed text should be indicated clearly on a manuscript. Each table and/or figure must have a legend that explains its purpose without reference to the text. Where a figure has more than one panel, each panel should be labelled in the top left-hand corner using lower case letters in parentheses i.e. ‘(a)’, ‘(b)’ etc., and

a brief description of each panel given in the figure legend. When using histology figures, the stain type and magnification level must be included in the legend.

Only figures of excellent quality will be considered for publication. The Journal will publish color photographs free of charge subject to editorial approval. When an individual is identifiable in a photograph written permission must be obtained (see Section 5 'Ethics' below).

Authors are themselves responsible for obtaining permission to reproduce previously published figures or tables.

References

References should be in Vancouver format and appear as consecutive, unbracketed superscript numbers in the text, e.g. 'in our previous reports[1,2] and those of Smith *et al*.[3–5]' and should be listed numerically in the reference list at the end of the article.

Format references as below, using standard (Medline) abbreviations for journal titles. When there are more than four authors, include the first three authors followed by *et al*.

1. de Berker DAR, Baran R, Dawber RPR. The Nail in Dermatological Diseases. In: *Baran and Dawber's Diseases of the Nails and their Management* (Baran R, Dawber RPR, de Berker DAR, Haneke E, Tosti, A, eds), 3rd edn. Oxford: Blackwell Science Ltd., 2001: 172–92.
2. Murray ML, Cohen JB. Mycophenolate mofetil therapy for moderate to severe atopic dermatitis. *Clin Exp Dermatol* 2007; **32**: 23–7.
3. Graham-Brown R, Burns T. *Lecture Notes: Dermatology*. Oxford: Wiley-Blackwell, 2006.
4. Smith A. (1999) Select committee report into social care in the community [WWW document]. URL http://www.dhss.gov.uk/reports/report015285.html [accessed on 7 November 2003].

5. DECLARATIONS

Original Publication

Submission of a manuscript will be held to imply that it contains original unpublished work and is not being submitted for publication elsewhere at the same time. The author must supply a full statement to the Editor about all submissions and previous reports that might be regarded as redundant or duplicate publication of the same or very similar work.

Conflicts of Interest

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