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High-frequency (20–50 MHz) ultrasonography of pseudoxanthoma elasticum skin lesions

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Summary

Background—In most patients pseudoxanthoma elasticum (PXE) manifests with yellowish cutaneous papules and dermal elastorrhexis on skin biopsy. However, in a small number of cases there are no skin manifestations on clinical examination. To establish a diagnosis of PXE in such patients with limited manifestations such as angioid streaks and/or premature cardiovascular disease is challenging. It would therefore be valuable to predict the skin areas that would yield a biopsy specimen positive for elastorrhexis with a non-invasive procedure. High frequency ultrasonography (HFUS) should be evaluated in this respect.

Objectives—Prior to achieve the goal mentioned above we aimed at describing the characteristics of clinically visible PXE skin using HFUS, and to evaluate its relevance for diagnosis.

Methods—HFUS was performed in a cohort of PXE patients and controls at a referral centre. HFUS images of PXE skin were compared to those of other conditions. Five operators were tasked with the blind scoring of multiple HFUS images of photoprotected or photoexposed skin from patients with PXE and controls. The diagnostic relevance indices (sensitivity, specificity, likelihood ratios, inter-observer agreement) were calculated.

Results—The HFUS changes considered as diagnostic for PXE were primarily oval homogeneous hypoechogenic areas in the middermis. The size of these areas closely matched the extent of the histological changes. The sensitivity and specificity of the diagnostic items and inter-observer agreement were high, particularly in photoprotected skin. Dermal hypoechogenicity in PXE could be related to high hydration of connective tissue due to the presence of glycosaminoglycans despite elastic fibre mineralization.

Conclusions—HFUS provides suggestive images of PXE skin lesions. HFUS should be now studied to determine if it is a potentially valuable technique for the non-invasive identification of elastorrhexis in PXE patients in whom skin involvement is clinically minimal or absent.

Keywords

Pseudoxanthoma elasticum; skin; ultrasonography; diagnostic test; elastic fibres; glycosaminoglycans

Pseudoxanthoma elasticum (PXE) is an uncommon autosomal recessive multisystem disorder affecting connective tissues (OMIM 264800). This monogenic disease is characterized pathologically by specific fragmentation and calcification of elastic fibres, primarily in the dermis, retinal Bruch's membrane and media of peripheral arteries. Indeed, skin lesions are often associated with retinal changes known as angioid streaks and early arteriosclerosis. All these manifestations are very heterogeneous, both in presentation and severity.^{1,2} The currently accepted criteria for the diagnosis of PXE are based on the combination of 1) clinical manifestations considered unique to PXE (yellowish dermal papules and plaques generally found on the main flexural regions of the body, i.e. the cervical, axillary, and groin areas, and the antecubital and popliteal fossae; angioid streaks), 2) histological elastic fibre changes referred to as elastorrhexis and 3) genetic markers (molecular diagnosis and/or family history).^{3,4}

The skin manifestations are present in most PXE patients, but they are either subtle or absent in a small number of cases.^{5–7} To establish a PXE diagnosis in patients with angioid streaks, premature cardiovascular disease and minimal or no skin involvement is challenging. The demonstration of elastorrhexis is therefore a critical step in the diagnostic process. It has been suggested that typical elastorrhexis can be evidenced in scars or even in skin of normal appearance.^{6,8} However, in our PXE referral centre, biopsies performed on non-lesional skin have often failed to demonstrate signs of elastorrhexis. Repeated and random skin biopsies would then be required but would certainly be viewed as too invasive by most patients.

Identifying the skin sites that are mostly likely to yield positive biopsy results would thus be valuable for clinical practice. We therefore hypothesized that high frequency ultrasonography (HFUS)^{9–11} might be a relevant non-invasive procedure to use for the detection of subclinical skin characteristics specifically inherent to PXE. Prior to achieving this goal, we first aimed at describing and evaluating the diagnostic significance of HFUS findings in PXE skin showing clinically visible changes.

Patients and methods

Patients

The PXE patients were recruited in our referral centre and diagnosed by the combination of suggestive skin changes, biopsy positive for elastorrhexis and angioid streaks on funduscopy. The controls were outpatients seen in the Dept. of Dermatology.

High frequency ultrasound (HF US) imaging of PXE skin lesions

Real-time 20–50MHz high resolution B-mode US imaging was performed with a DERMcup® 2020 (Atys Medical, Soucieu, France) by two investigators (MGM and GL). The device was used with axial and lateral resolution of 80µm and 200µm, respectively, and a field of view of 6×5 mm (width × depth). The skin surface was scanned manually on 2 different types of skin frequently involved in PXE: the sun-exposed nape of the neck and the photoprotected flexural and/or periumbilical skin that presumably did not contain significant actinic elastic fibre damage. PXE patients from our cohort were prospectively examined

with the DERMcup® 2020 to identify HFUS echostructures unique to PXE. The procedure was repeated with unaffected individuals by the same investigators and the findings were used as controls. This study has been approved by the institutional review board of Angers University and consent has been obtained from all participants.

PXE-specific pathology findings

To support that the HFUS results were related to PXE-specific pathological changes, we compared the physical characteristics of dermal changes observed with HFUS with microscopic changes on paraffin-embedded samples. We anticipated that we would be unable to make a direct HFUS / microscopy comparison in all PXE patients included in the study. Indeed, 1) many of them had already had a biopsy diagnosis of PXE before the beginning of this study, 2) the remaining ones underwent a small punch biopsy only covering a single skin papule. Therefore, we studied 3 additional patients who underwent plastic surgery to remove loose axillary skin. Of note, we examined the edges of the large surgical skin fragments to specifically evaluate the papular skin lesions (round dermal areas with elastorrhexis) rather than the regions with severe loss of elasticity (dermal band-like elastorrhexis). Pathology evaluation included usual H/E and orcein stainings as well as Von Kossa and Alcian blue stainings to demonstrate elastic fibre calcification and glycosaminoglycans deposition in the dermis, respectively.

Diagnostic relevance indices and inter-observer correlation

Five operators were tasked with the blind scoring of multiple consecutive HFUS images as either « PXE » or « NOT PXE » using the diagnostic criteria detailed below. A random set of 25 images that included sun-exposed nuchal skin from 15 PXE patients was scored first, followed by a second set of 24 images of photoprotected skin (antecubital fossa or periumbilical skin) that included 11 PXE patients. The investigators were blind to the diagnosis of PXE and the number of patients in both sets of images. Diagnostic relevance indices (sensitivity, Se, specificity, Sp, likelihood ratios, LR) and inter-observer agreement (Kappa coefficient) were calculated after all scoring had been performed.

Statistical analyses

All variables were expressed as mean $\pm$ SD. For all quantitative variables, comparisons of continuous variables were performed using the non-parametric Wilcoxon test for unpaired samples. Comparisons between the dichotomous variables were performed using the Fisher exact test. All statistical analyses were performed with STATA 12.0 (StataCorp, College Station, TX, USA,) and significance was set at $p < 0.05$. Comparisons of Kappa coefficients, sensitivity, specificity and likelihood ratios were performed using boot strap-based tests.

Results

High frequency ultrasound (HF US) imaging of PXE skin lesions

After examination of more than 30 PXE patients in a year, the following HFUS criteria were considered as diagnostic of a PXE skin papule:

- undulating skin surface with normal epidermis and dermal/epidermal interface,
- oval homogeneous hypoechogenic area in the mid and deep dermis, and
- echoic interpapular dermis (Fig. 1–3).

Twenty and 50 MHz HF US provided similar findings.

Comparison of pathology and ultrasonography findings in lesional PXE skin

One third of the patients enrolled underwent a diagnostic biopsy during the study. In all of them we observed a clear matching between HFUS changes and elastorrhexis. Forty-nine “nodules” were measured on skin sections from the 3 additional PXE patients who underwent surgery. “Nodule” average dimensions were $2.87 \pm 1.39 \text{ mm} \times 0.94 \pm 0.48 \text{ mm}$ (Fig. 4). On HFUS, oval homogeneous hypoechogenic area dimensions were $1.84 \pm 0.71 \text{ mm} \times 0.97 \pm 0.29 \text{ mm}$ for the nuchal area, and $1.73 \pm 0.63 \text{ mm} \times 0.98 \pm 0.26 \text{ mm}$ for photoprotected skin. The HFUS dimensions of oval homogeneous hypoechogenic areas from photo-exposed and photo-protected areas did not differ significantly.

Diagnostic relevance indices and inter-observer agreement

For each PXE patient enrolled in the study, HFUS images were recorded with up to 6 dermal oval homogeneous hypoechogenic areas. In total, 48 and 47 oval homogeneous hypoechogenic areas were studied for photoexposed and photoprotected skin, respectively. As comparative controls, we used HFUS images obtained in known disease states such as actinic elastosis (9 cases with *cutis rhomboidalis nuchae*), other connective tissue diseases (Ehlers-Danlos syndromes), and various papular skin neoplasms as well as normal skin (Fig. 5). All the variables studied are presented in Table 1. Briefly, HFUS diagnostic items were both sensitive and specific. However, sensitivity and specificity were higher for the photoprotected skin than for the photoexposed skin ($\text{Se} = 89.5\%$ (95% CI = 78.5–96.0) vs 86.2% (95% CI = 75.3–93.5), $p = 0.365$; and $\text{Sp} = 93.7\%$ (95% CI = 84.5–98.2) vs 68.3% (95% CI = 55.0–79.7), $p = 0.008$, for photoprotected vs photoexposed skin, respectively). The positive likelihood ratio was higher for the protected skin than for the nuchal skin ($\text{LR}+ = 14.1$ (95% CI = 5.44–36.5) vs 2.72 (95% CI 1.85–4.00) – $p = 0.004$, and the negative likelihood ratio was not significantly lower for the photoprotected skin than for the nuchal skin ($\text{LR}- = 0.112$ (95% CI = 0.053–0.240) vs 0.203 (95% CI = 0.108–0.381), $p = 0.778$). The inter-observer agreement was also better for photoprotected skin (Kappa = 0.799 (95% CI = 0.630–0.969)) than for photoexposed skin (Kappa = 0.407 (95% CI = 0.226–0.625)), $p = 0.003$.

Discussion

In this report we described the unique subepidermal echostructure characteristics of the skin of individuals affected by PXE revealed by HFUS. One of our main finding was that, contrary to our expectation, PXE skin was primarily hypoechogenic.

In recent years new tools have been developed to improve the accuracy of diagnosis in dermatological practice. Dermoscopy can provide useful additional findings to clinical examination and can help in reducing the need of a biopsy. However, dermoscopy only shows the surface of the skin. Ultrasonography is widely used by radiologists to visualize subcutaneous structures. The frequency of 5–7 MHz allows visualization of deeply located organs (e.g. liver, kidney) and the range between 7 and 13 MHz is necessary for more superficial tissues (muscle, breast...). However, the resolution is not sufficient for dermatology purposes. Therefore, HFUS systems with frequencies over 20 MHz and a marked increase in resolution now offer an improved visualization of skin changes. HFUS provides certain information that cannot be provided by dermoscopy, i.e. the general shape of a lesion, whether the structure is hypoechoic or not, homogeneous or not, whether the borders can easily be distinguished from adjacent structures or not etc...^{9–11}

The ultrasound structure of PXE skin was expected to be hyperechogenic because of the calcification of the dermal elastic fibres. However, no significantly echogenic structure was evidenced in skin with visible PXE lesions. Actinic skin damage results in elastolysis in the

papillary dermis revealed on histologic examination and in a subepidermal non-echogenic band (SENEB) in HFUS (Fig. 5).¹² Thus, the fragmentation of elastic fibres in elastorrhexis might cause *per se* the hypoechogenic structure in PXE lesional skin, with lack of dermal echoes due to the small size of calcifications insufficient to generate echoes. Another explanation for our results could be that the dermal hypoechogenicity of PXE resulted from a higher level of hydration of the PXE connective tissue. Naouri et al. recently showed that skin oedema associated with lymphoedema was responsible for decreased echogenicity. Interestingly, in their study hypoechogenicity increased from the thigh to the ankle, in complete compatibility with clinical findings since the distal portion of the lower limb is more severely affected than the proximal.¹³ There is no obvious sign of oedema in PXE, though the abnormal presence of glycosaminoglycans in PXE skin may explain the apparently high hydration status we inferred from HFUS observations.^{14–16} Further, our histological findings clearly support the presence of large deposits of glycosaminoglycans in a close association with calcified elastic fibres (Alcian blue staining) (Fig. 4). These findings are also consistent with the arterial characteristics in PXE. Kornet et al. reported greater elasticity of the carotid artery in PXE patients than in control individuals. This result was attributed to deposition of glycosaminoglycans in addition to elastin fragmentation in the media, despite the presence of mineralization.¹⁷

The HFUS ultrastructure of the PXE skin lesions featuring oval homogeneous hypoechogenic areas was unique in our experience and closely matched the findings made with the paraffin-embedded samples with respect to overall morphology and dimensions. The slight differences seen on the fixed samples were most probably due to the more severe skin manifestations in the patients that required corrective surgery. We conclude from this study that HFUS was demonstrated to be both sensitive and specific as a complementary diagnostic tool, particularly in photoprotected areas. This approach appears advantageous for it does not require a high level of expertise and allows easy discrimination between PXE and other common skin changes, including dermal elastosis and age-related changes (subepidermal non- or hypoechogenic band) (Fig. 5).¹² The PXE echostructure was also different from other connective tissue diseases studied with HFUS, and could be used for differential diagnosis in ambiguous cases. Several publications have stated that dermal thickness in classical and hypermobile forms of Ehlers-Danlos syndrome is reduced^{18–20} although dermal echogenicity is homogeneous.

In summary, we observed a strong correlation between the HFUS PXE characteristics and the severity of the skin changes. Because undisputable elastorrhexis has been previously observed in absence of visible skin lesions^{6,8} we suggest the use of HFUS for the non-invasive identification of the skin characteristics unique to PXE, particularly in sun-protected skin. We already have successfully used HFUS in the diagnosis of several PXE patients with angioid streaks and no clinically visible skin changes or changes of unclear significance (Fig. 6). However, further investigations including a greater number of patients will be needed to clarify this point and to confirm our results.

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Bulleted statements**What's already known about this topic?**

Pseudoxanthoma elasticum (PXE) skin changes typically are yellowish papules and are associated with unique elastic fibre fragmentation and calcification on skin biopsy. High frequency ultrasonography features of cutaneous PXE have never been reported.

What does this study add?

Non-invasive high frequency ultrasonography examination of the skin of PXE patients revealed unique echostructure characteristics with oval homogeneous hypoechogenic areas in the middermis. These findings may be used for diagnosis of PXE in ambiguous cases. They should be studied now to optimize the choice of skin site for biopsy in patients with subtle or no clinical changes. Last, the hypoechogenic areas identified were consistent with the presence of localized hydration due to deposits of glycosaminoglycans.

Figure 1. High-frequency ultrasonography of the skin (50 MHz). Periumbilical PXE skin lesions in a 70-year-old woman. Note the oval hypoechogenic areas (*), suggestive of PXE.

Figure 2. Antecubital papules of PXE in a 60-year-old woman with corresponding hypoechogenic dermal areas (*) on 20 MHz HFUS examination.

Figure 3. PXE papules on the nuchal area of a 17-year-old girl. Compare the ovoid hypoechoic areas (*) with the normal HFUS structure of perilesional unaffected dermis (^).

Figure 4.

A. Large fragment of PXE skin (surgical sample) demonstrating dermal “nodules” of elastorrhexis (Von Kossa staining. Original magnification x20). B. Alcian blue staining of dermal elastorrhexis: large deposits of glycosaminoglycans are in close association to calcified elastic fibres but spare other fibres (Original magnification x300)

Figure 5.

HFUS images of control skin diseases used in the study. A : actinic elastosis and skin aging of the forearm - characteristic subepidermal non-echogenic band (SENEB) (*) (HFUS 50 MHz). B : hypermobile Ehlers-Danlos syndrome in a 23-year-old woman -reduction of thigh skin thickness but homogeneous dermal echogenicity (HFUS 20 MHz). C : chronic leg lymphoedema with dermal oedema and related hypoechogenicity (HFUS 50 MHz)

Figure 6.

Diagnosis advantages of HFUS in PXE.

Panel A: Absence of visible cutaneous changes on the upper aspect of the thigh (a) in a 13-year-old girl with ocular peau d'orange and angioid streaks. 50 MHz HFUS identified discrete hypoechogenic areas (*) in the dermis (b). The biopsy oriented by HFUS revealed calcification of elastic fibres without dystrophy (von Kossa, original staining x300) (c). These pathological changes are likely indicative of nascent lesions of PXE. *ABCC6* genotyping later revealed two pathogenic mutations.

Panel B: Small papules on the antecubital fossa (a) of a 59-year-old woman with known PXE. Such changes are suggestive but not specific to PXE. 50 MHz HFUS scans identified a single hypoechogenic area (*) in the dermis of this patient (b). The biopsy at this site demonstrated undisputable elastorrhexis (von Kossa, original staining x75) (c).

Table 1

Diagnostic indices and inter-observer agreement (CI 95%)

Photoexposed group	Photoprotected group	p
Se = 86.2% [75.3–93.5]	Se = 89.5% [78.5–96.0]	0.365
Sp = 68.3% [55.0–79.7]	Sp = 93.7% [84.5–98.2]	0.008
LR+ = 2.72 (95% CI = 1.85–4.00)	LR+ = 14.1 (95% CI = 5.44 –36.5]	0.004
LR– = 0.203 (95% CI = 0.108–0.381)	LR– = 0.112 (95% CI = 0.053–0.240)	0.778
Kappa = 0.407 [0.226–0.625]	Kappa = 0.799 [0.630–0.969]	0.003

CI = confidence interval

Se = sensitivity

Sp = specificity

LR+ = positive likelihood ratio, LR– = negative likelihood ratio

Kappa = inter-observer agreement index