The endocardial binary appearance (‘binary sign’) is an unreliable marker for echocardiographic detection of Fabry disease in patients with left ventricular hypertrophy

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Aims
The binary sign, a binary appearance of the left ventricular endocardial border, was suggested to be an echocardiographic hallmark in diagnosing Fabry disease, a hereditary, lysosomal storage disorder. The aim of the present study was to examine the reliability of the binary sign as a screening tool to identify patients with Fabry disease.

Methods and results
In total 309 subjects with an interventricular septum (IVS) thickness of ≥ 12 mm were investigated, of which 14 had a confirmed diagnosis of Fabry disease. Urinary globotriaosylceramide testing was used to rule out Fabry disease in the control group. From all patients echocardiographic images of the apical four-chamber view were analysed offline by a blinded observer. A binary sign was seen in 63 patients (20%), 4 had Fabry disease and 59 belonged to the control group. Although the proportion of binary signs in patients with Fabry disease was higher (29%) compared with the control group (20%) this difference was not statistically significant. The sensitivity and specificity were 28% (95% confidence interval (CI): 12–65%) and 80% (95% CI: 76–85%), respectively. In a logistic regression model adjusted for age, sex and presence of Fabry disease, the occurrence of a binary sign was highly dependent on the IVS thickness (odds ratio: 1.21; 95% CI: 1.1–1.35; P < 0.001).

Conclusion
The endocardial binary appearance is associated with the degree of septal hypertrophy but cannot adequately distinguish between patients with Fabry disease and patients with other causes of left ventricular hypertrophy.

Keywords
Fabry disease • Binary sign • Left ventricular hypertrophy • Hypertrophic cardiomyopathy

Introduction
Fabry disease is a rare, hereditary, X-linked lysosomal storage disorder with accumulation of globotriaosylceramide (Gb3) in various organs including the heart. Progressive myocardial hypertrophy frequently develops over the years and is the most common cardiac pathology in Fabry disease, leading to ventricular diastolic and systolic dysfunction. Valvular functional abnormalities and arrhythmias are additional cardiac manifestations responsible for premature death in these patients.1–4 Since enzyme replacement therapy seems to influence the cardiac impairment positively, correct diagnosing of Fabry disease also gains importance in Cardiology.

Fabry disease was associated in up to 12% in female2 and up to 6% in male subjects3 with late-onset hypertrophic cardiomyopathy.

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However, the severity of left ventricular hypertrophy in Fabry disease is variable and cardiac abnormalities are rather unspecific.

Recently, Pieroni et al. described a binary appearance—the so-called ‘binary sign’—of the left ventricular endocardial border, mainly manifested at the interventricular septum (IVS) in the four-chamber view in Fabry patients. In this study, the binary sign was suggested to be a diagnostic hallmark enabling echocardiographic diagnosis of Fabry disease.

The aim of the present study was to examine in a large cohort of patients the diagnostic performance of the binary sign potentially providing an echocardiographic tool to distinguish left ventricular hypertrophy caused by Fabry disease from other etiologies of left ventricular hypertrophy.

**Methods**

**Study population**

Out of the population of the ‘Viennese Prevalence Study of Anderson-Fabry Disease’ (VIEPAF; ClinicalTrials.gov identifier: NCT00871611) in patients with left ventricular hypertrophy (IVS thickness ≥12 mm) a random sample of 295 subjects together with confirmed patients with Fabry disease (n=14) with an IVS thickness ≥12 mm were evaluated for the occurrence of a binary sign. Basic demographic data were collected at the most recent available examination date.

All subjects from the VIEPAF study gave informed consent and the institutional review committee approved the study.

**Echocardiography**

Echocardiographic studies were performed with Vivid 7 (GE, Vingmed Ultrasound AS, Horten, Norway) or Acuson Sequoia C512 (Acuson, Inc., Mountain View, CA, USA). For image acquisition, harmonic imaging with 3.5 MHz transducers with different gain settings was used.

The binary sign was defined as suggested by Pieroni et al. as a hyper-echogenic and bright endocardium and a hypoechogenic space between endo- and myocardium, especially visible in the interventricular septal and the apical region of the left ventricle, respectively, thus forming a binary appearance.

Echocardiographic images were digitally stored and analysed offline by three blinded observers on Echopac (GE) workstations. All available four-chamber apical view images, which were collected at different examination dates, were evaluated. First, all images were reviewed in original format. Image modification by changes of gain settings is usually performed during offline analysis to improve image quality. At the end of the review process, the observer had to judge if a binary sign was present for each patient. To assess interobserver agreement in a sub-analysis three different observers (A, B, and C) independently evaluated 62 subjects (all out of the main analysis) for a binary sign containing 14 patients with Fabry disease and 48 control subjects with left ventricular hypertrophy of any cause. To assess intraobserver variability, observer A re-evaluated these 62 subjects 2 months later.

**Laboratory methods**

To exclude Fabry disease, all control subjects underwent a urinary screening test for Gb3 (Gb3 24:18 ratio >2.3 rise suspicion for the presence of Fabry disease). For patients with a urinary Gb3 concentration above the critical cut-off, α-galactosidase-A activity was tested and GLA-mutation analysis was performed as previously described.

**Statistical analysis**

Continuous data are described by mean ± standard deviation (SD) or median and inter-quartile range (IQR), categorical data are presented

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**Figure 1** Study population. VIEPAF, Viennese Anderson-Fabry disease screening in patients with left ventricular hypertrophy; IVS, interventricular septum.
as count and percentage. Groups were compared using the Mann–Whitney U test and Fisher’s exact test or the Pearson $\chi^2$ test, respectively. A logistic regression model with age, sex, presence of Fabry disease, and IVS thickness was estimated to evaluate which of these factors might predict the presence of a binary sign $[\text{OR} = \text{odds ratio}; 95\% \text{ confidence interval (CI)}]$. Both interobserver and intraobserver agreement were determined with a kappa ($k$) statistic. Kappa values from 0.4 to 0.59 are considered moderate, 0.6–0.79 substantial and 0.8 outstanding as suggested by Landis and Koch. $P$-values $<0.05$ were considered as indicating statistical significance. PASW Statistics 18 software was used for statistical computations.

## Results

The patient disposition is highlighted in detail in Figure 1. The control group (out of the VIEPAF study patients) consisted of 295 patients with left ventricular hypertrophy (IVS thickness $\geq 12$ mm), which were referred to our outpatient service with clinical diagnoses of valvular disease ($n = 99$), coronary artery disease ($n = 54$), cardiomyopathy ($n = 31$; 23 hypertrophic cardiomyopathy), hypertension ($n = 29$), heart transplantation ($n = 8$), arrhythmias ($n = 21$), and other diagnoses ($n = 53$). For 21 subjects who had a positive Gb$_3$-urinary screening test, enzymatic and genetic testing were negative for Fabry disease.

Of 23 subjects with hypertrophic cardiomyopathy, 12 presented with left ventricular outflow tract obstruction. The pattern of hypertrophy was variable (five patients concentric, six patients asymmetric, three patients with hypertrophy localized to apical and septal regions, and nine patients with variable distribution of hypertrophy).

For 25 Fabry patients under medical treatment at the Vienna General Hospital, echocardiographic studies were available. Only 14 presented with an IVS thickness $\geq 12$ mm and were therefore included in our analysis. Three of the 11 excluded subjects revealed a binary sign, although the IVS thickness was only 11 in 2 cases and 9 in the third. The pattern of hypertrophy was concentric in all Fabry patients with advanced left ventricular hypertrophy. No patient presented with left ventricular outflow tract obstruction.

The patient’s characteristics are given in Table 1. A binary sign (Figure 2) was seen in 63 patients (20%), 4 had Fabry disease and 59 belonged to the control group. Although the proportion of binary signs in the Fabry group was higher (29%) compared with the control group (20%) this difference was not statistically significant (95% CI for difference in proportions: $-16\%$ to $33\%$; $P = 0.661$). We calculated a sensitivity of 29% (95% CI: 12%–65%) and a specificity of 80% (95% CI: 76%–85%).

![Figure 2](image_url) The endocardial binary appearance of the left ventricle (apical four-chamber view) in a patient with Fabry disease (A) and without Fabry disease (B).

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Patient characteristics [mean ($\text{± SD}$) or count (%)]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fabry disease</td>
</tr>
<tr>
<td>$n$</td>
<td>14</td>
</tr>
<tr>
<td>Age (years)</td>
<td>46 ($\text{± 15}$)</td>
</tr>
<tr>
<td>Female</td>
<td>6 (43)</td>
</tr>
<tr>
<td>IVS thickness (mm)</td>
<td>15.2 ($\text{± 2.8}$)</td>
</tr>
<tr>
<td>Binary sign (yes)</td>
<td>4 (29)</td>
</tr>
<tr>
<td>IVS thickness $\geq 15$ mm</td>
<td>8 (57)</td>
</tr>
<tr>
<td>IVS thickness $\geq 15$ mm and binary sign</td>
<td>2 (25)</td>
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IVS, interventricular septum.
binary sign (OR: 1.21; 95% CI: 1.1–1.35; \(P\), 0.001), emphasizing the relation between hypertrophy and the likelihood of observing a binary sign. The association between hypertrophy and a binary sign remained significant even after exclusion of the 14 patients with Fabry disease (OR: 1.21; 95% CI: 1.1–1.35; \(P\), 0.001).

One hundred and thirty-nine subjects were examined using the Acuson Sequoia C512 ultrasound system and 102 were evaluated by the GE Vivid 7. We diagnosed a binary sign in 24 (17%) subjects of the former group, but in 29 (28%) in the latter (\(P\), 0.04), leading to the assumption that it may be more likely to see a binary sign when the GE Vivid 7 Ultrasound system is used.

To determine the interobserver agreement, observers A, B, and C evaluated independently 63 subjects, a subsample taken out of the main analysis consisting of 14 Fabry patients and 49 controls with left ventricular hypertrophy. In seven cases two observers diagnosed a binary sign when the third could not see the binary appearance of the IVS. In 10 cases, only one observer was confident that a binary sign was present. This resulted in a poor interobserver agreement of 43% between A and B, 48% between B and C, and 65% between A and C. However, for the main analysis no adjustment has been undertaken regarding the discrepancies, because the overall sensitivity and specificity did not differ between the three observers (A: 29 and 73%; B: 29 and 86%; C: 36 and 73%). Finally, the intraobserver variability was 81% computed for observer A.

**Discussion**

Finding a simple, yet accurate way of diagnosing Fabry disease still remains a puzzle. From the variety of the clinical course of this rare storage disorder, one may infer that undiagnosed cases are spread out over outpatient services. Enzyme replacement therapy showed significant improvement in clinical symptoms of Fabry patients. Therefore, an early identification of affected subjects gains importance.

In routine echocardiography frequently observed morphologic pathologies, as left ventricular hypertrophy and myocardial or valvular functional changes, are unspecific and do not allow to distinguish Fabry disease from other causes of left ventricular hypertrophy. Pieroni et al. first provided a possible morphological explanation of the binary appearance of the left ventricular endocardial border in echocardiographic studies. In 20 (100%) Fabry patients with distinct left ventricular hypertrophy (maximal wall thickness >15 mm) and in 13 (65%) Fabry patients with a maximal wall thickness ≤15 mm the binary sign was present. Remarkably, in this study none of the control group, consisting of patients with hypertrophic cardiomyopathy (\(n\) = 40),
hypertensive patients with increased septal thickness (n = 40) and healthy controls (n = 40), presented with a binary sign. The binary sign was interpreted as to be the ultrasound image of ‘an inner glycolipid-rich layer including endocardium and severely affected myocardium, and an outer layer represented by a mildly affected myocardium corresponding to the midwall portion of ventricular wall’. The sensitivity and specificity were 94 and 100%, respectively, apparently rendering the binary sign an outstanding and easy assessable tool to identify Fabry patients.

In total contradiction to the above-mentioned study, in our study population only 4 out of 14 Fabry patients presented with a binary sign, whereas in 59 of 295 subjects of the control group a binary sign was observed as well. These findings resulted in a poor sensitivity and specificity. Notably, the IVS thickness was associated with the occurrence of a binary sign in both, patients with Fabry disease and controls.

Kounas et al. examined 14 age- and gender-matched patients with confirmed Fabry disease, of which 86% had left ventricular hypertrophy. The control group comprised 14 patients with hypertrophic cardiomyopathy defined as IVS thickness ≥15 mm or if IVS thickness was <15 mm the diagnosis was based on familial diagnostic criteria. They found a sensitivity of 35% and a specificity of 79%. With an increased left ventricular wall thickness sensitivity raised (44%) at the expense of specificity (70%). It was concluded that the binary sign cannot reliably discriminate Fabry disease from other causes of hypertrophic cardiomyopathy.

Recently, Koskenvuo et al. analysed 23 echocardiographic studies of 13 patients with Fabry disease and 15 matched control patient studies. Four control patients presented with a binary sign, but only in two Fabry patients the sign was found, of which one had left ventricular hypertrophy.

The histomorphological concept of Pieroni et al. depicted as the binary sign by ultrasound promised a simple and early identification of Fabry patients with cardiac impairment. However, phenotypic variation across Fabry patients is substantial and the morphologic cardiac changes are heterogeneous as well. Moreover, it is known that Gb3 accumulation within the myocardium does not correlate with the severity of left ventricular hypertrophy. Hence, morphologic changes may not only vary across individual Fabry patients, but the affection of the myocardial tissue might also be inhomogeneous within one patient. The previously described definition of the binary sign lacks precise description allowing a wide scope of interpretation. Thus, reliable judgement may fail due to subjectivity. Importantly, it remained open, whether different echocardiographic views should be taken into account or if the presence of a binary sign should be strictly examined in the four-chamber view. For reasons of comparability among the three observers, we primarily evaluated the occurrence of a binary sign in the four-chamber view. Although disregarded in the main analysis, the observers also looked at the three- and two-chamber views. In some cases the finding could be observed in the four-chamber apical view, but not in other views and vice versa, implying that it might not only be the suggested histological phenomenon. In addition, we saw that the determination of the binary sign depends on the echocardiographic sectional plane and is observer dependent and not always reproducible in repeated examinations. However, when looking at consistency of the presence or absence of the binary sign within one subject over time only in one patient with Fabry disease the opinion of the observer was clearly divided. In the end, the respective case was judged as binary sign negative, because the impression of a binary sign faded in further investigations.

These issues are in line with the poor interobserver agreement observed in the present study, although intraobserver agreement was 81%. By comparison, Kounas et al. found an inter- and intraobserver agreement of 44 and 35%, respectively, while Koskenvuo et al.10 reported 84% for interobserver agreement and 87% for intraobserver agreement. Notably, Pieroni et al. did not investigate these crucial facts in their study at all.

Several factors, such as acquisition modes and gain settings, may influence the appearance of the binary sign, poor image quality in general limits the evaluation of echocardiographic studies. Moreover, the possibility to judge using echocardiographic studies of different ultrasound systems might lead to an even more heterogeneous picture. The poor reproducibility and the variability caused by ultrasound settings and various software systems were also observed by Kounas et al. and Koskenvuo et al. who did not change gain settings throughout the analysis.

The major strength of our study is the large sample size. The heterogeneous control group displays a typical variety of cardiac patients one has to face in an echocardiography outpatient service, thus mimicking routine diagnostic settings. Notably, we...
excluded unknown Fabry disease in all control patients, in contrast to all the other studies discussed before.

**Limitations**

In this study patients with Fabry disease and an IVS thickness <12 mm were excluded, since the control group exclusively consisted of patients with an IVS thickness ≥12 mm. However, three patients with Fabry disease and an IVS thickness <12 mm presented with a binary sign. The question whether the binary sign can discriminate patients with Fabry disease without left ventricular hypertrophy from patients with normal wall thickness has not been examined in our study and remains open.

Yet, no specific hallmark for echocardiographic detection of Fabry disease is known. Patients with Fabry disease may show a variety of morphologic changes. The pattern of hypertrophy is usually concentric, however, there are several cases described with asymmetric pattern or additional outflow tract obstruction. Kounas et al. showed that left ventricular end-diastolic diameter is significantly higher in patients with Fabry disease compared with patients with hypertrophic cardiomyopathy. Furthermore, cases of Fabry disease are reported, which mimic amyloidosis in two-dimensional echocardiography. We did not examine whether the binary sign is associated with other morphologic changes. Also, we did not imply clinical information as proposed by several authors for screening patients with unexplained hypertrophy. Taking into account these limitations, the sensitivity of the binary sign as a diagnostic tool may have been increased in our study.

**Conclusion**

The echocardiographic finding of a binary appearance of the left ventricular endocardial border is a morphologic feature, which is lacking accurate definition, highly operator dependent and therefore poorly reproducible. It is significantly associated with the severity of left ventricular hypertrophy of variable causes including Fabry myopathy. Thus, we conclude that the binary sign is not helpful in a daily routine setting for echocardiographic identification of Fabry disease in patients with left ventricular hypertrophy.

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