Characterizing the Natural History of Visual Function in Choroideremia Using Microperimetry and Multimodal Retinal Imaging

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Submitted: June 24, 2017
Accepted: September 18, 2017
Citation: Jolly JK, Xue K, Edwards TL, Groppe M, MacLaren RE. Characterizing the natural history of visual function in choroideremia using microperimetry and multimodal retinal imaging. Invest Ophthalmol Vis Sci. 2017;58:5575–5583. DOI:10.1167/iovs.17-22486

PURPOSE. Centripetal retinal degeneration in choroideremia (CHM) leads to early visual field restriction and late central vision loss. The latter marks an acute decline in quality of life but visual prognostication remains challenging. We investigated visual function in CHM by correlating best-corrected visual acuity (BCVA), microperimetry and multimodal imaging.

METHODS. Fifty-six consecutive CHM patients attending Oxford Eye Hospital were examined with BCVA, 10–2 microperimetry, optical coherence tomography, and fundus autofluorescence (AF). Microperimetry was repeated in 21 eyes and analyzed with Bland-Altman. Kaplan-Meier survival plots of eyes retaining 20/20 BCVA were created. Intereye symmetry was assessed.

RESULTS. Microperimetry coefficient of repeatability was 1.45 dB. Survival analysis showed an indistinguishable pattern between eyes (median survival 39 years). Macular sensitivity showed a similar decline in right and left eyes, with half-lives of 13.6 years. Zonal analysis showed faster decline nasal to the fovea. Intereye symmetry was more consistent for microperimetry sensitivity ($r = 0.95, P < 0.001$) than BCVA ($r = 0.42, P = 0.0006$). Near normal foveal sensitivity was maintained when the fovea was at least 2500 μm from the advancing edge of AF.

CONCLUSIONS. BCVA is a marker of central degeneration and can provide valuable information about the position of the remaining retina as well as a measure of the impact on daily living. Microperimetry represents the global macular region. Both visual functions showed a high degree of intereye symmetry, particularly in early stages, indicating the fellow eye can provide a suitable control for assessing interventions to one eye. The findings may help to tailor visual prognosis and interpret outcomes of trials.

Keywords: choroideremia, microperimetry, structure-function analysis, fundus autofluorescence

Choroideremia (CHM) is a progressive X-linked inherited retinal degeneration affecting the RPE, choroid, and outer retina. It is caused by a mutation of the CHM gene, which encodes Rab escort protein-1 (REP1), a key mediator of membrane trafficking in the photoreceptors and RPE. Early symptoms include nyctalopia and reduced peripheral vision, which typically proceeds to loss of visual acuity (VA) and legal blindness as early as the third or fourth decade. With the advent of gene therapy as a possible treatment for CHM, it is important to establish reliable functional outcome measures for use in clinical trials.

Microperimetry is a static automated visual field test which uses real-time fundus scanning laser ophthalmoscopy (SLO) and an eye position tracker to map threshold sensitivities at specific and customizable retinal loci within the macular region. Threshold light stimuli of varying intensity are displayed on a mesopic background to test visual sensitivity in the central 10 degrees of the macula. The method can be used to delineate small scotomas, to assess changes in fixation stability, and to identify general or specific areas of threshold change in different disease conditions. It is being increasingly used to monitor a variety of conditions including CHM, achromatopsia, AMD, and diabetes. It has been correlated to contrast sensitivity as well as to optical coherence tomography (OCT) appearance. The anticipated potential benefits of using microperimetry as an outcome measure in CHM gene therapy clinical trials include accurate threshold sensitivity mapping at individual retinal points over time (e.g., in the region exposed to a subretinal delivery of viral vector), and automated adjustments for eye movements that may compensate for fixation losses related to poor VA. It is also a valuable technique to inform patients about their disease progression in a clinical setting.

Although an X-linked retinal dystrophy would be expected to affect both eyes to a similar extent in males, it is unclear whether visual functions declined symmetrically in choroideremia, an important consideration when designing clinical trials in which the fellow eye may be used as a control for the treated eye.
eye. The characteristic centripetal pattern of outer retinal degeneration of the central retinal island in CHM can be imaged using fundus autofluorescence (AF) and OCT, thereby providing a unique opportunity to study the correlation between photoreceptor/RPE structural integrity and visual function. The aim of this work was to assess the utility of VA, Macular Integrity Assessment (MAIA) microperimetry, and multimodal retinal imaging in a large cohort of patients with choroideremia. The characteristic centripetal pattern of outer retinal degeneration of the central retinal island in CHM can be imaged using fundus autofluorescence (AF) and OCT, thereby providing a unique opportunity to study the correlation between photoreceptor/RPE structural integrity and visual function. The aim of this work was to assess the utility of VA, Macular Integrity Assessment (MAIA) microperimetry, and multimodal retinal imaging in a large cohort of patients with choroideremia.
CHM, to provide a better understanding of the intereye symmetry and natural history of disease progression with age.

Masking of eyes is the gold standard for intravitreal treatments, such as with anti-VEGF therapies, whereby a sham procedure can justifiably be performed in a nontherapy arm of the clinical trial. With CHM gene therapy, however, this is not ethically acceptable, because the subretinal injection is complex and not without risk. Furthermore, the gene therapy procedure takes 2 hours under general anesthesia, with the patient taking systemic prednisone. Arguably, therefore, the fellow eye may present a more ethically acceptable control. This could be achieved with a long-acting anesthesia (such as bupivacaine) and atropine given to dilate and blur the fellow eye. Because VA recovery is generally achieved within 1 week following CHM gene therapy, this might be a viable option. However, to use the fellow eye as a control, it is first necessary to define the parameters of symmetry between eyes in affected patients. Hence this was the purpose of the current study.

**METHODS**

Patients were assessed as part of an ongoing clinical trial, an open-label Phase 2 clinical trial of retinal gene therapy for CHM using an adeno-associated viral vector encoding REP1 (ClinicalTrials.gov identifier: NCT02407678, approved by National Research Ethics Service Committee London–West London and Gene Therapy Advisory Committee [GTAC], reference GTAC171), conducted in accordance with the Declaration of Helsinki at the Oxford Eye Hospital, UK. The cohort comprised patients with confirmed mutations within the CHM gene.16 112 eyes from 56 consecutive patients were included in the analysis. Patients undertook measurements of best-corrected VA (BCVA) followed by macular sensitivity using the MAIA microperimeter (Centervue SpA, Padova, Italy) after 20 minutes of dark adaptation (light level < 1 lux). Patients who reported amblyopia in one eye were not included, as per the trial protocol. All microperimetry field plots had a reliability score ≥ 80% as determined by blind spot testing. The test configuration for all patients was a 10–2 grid (with 4–2 bracketing strategy) centered on the fovea, as shown in Figure 1. This is similar to a Humphrey 10–2 test protocol.17

After visual function testing, both eyes were dilated with 1% tropicamide and 2.5% phenylephrine. Spectral-domain OCT (SD-OCT) and 30° autofluorescence (AF) imaging (automatic real-time mode, averaging a minimum of 25 images) were performed on the Heidelberg Spectralis (Heidelberg Engineering GmbH, Heidelberg, Germany). The area of residual AF was measured using the Heidelberg Eye Explorer (HEYEX) software area tool as per previously published methodology.13 The anatomical foveola as seen on the OCT was mapped to the corresponding location on the AF image using technique

### Table. Summary Statistics for Patient Groups

<table>
<thead>
<tr>
<th>Factor</th>
<th>Full Cohort</th>
<th>Repeatability Cohort</th>
<th>Reduced Threshold Cohort</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients, n</td>
<td>56</td>
<td>21</td>
<td>33</td>
</tr>
<tr>
<td>Age, y, mean ± SD (range)</td>
<td>37.8 ± 16.9 (12–74)</td>
<td>34.1 ± 13.3 (12–70)</td>
<td>47.9 ± 14.9 (18–74)</td>
</tr>
<tr>
<td>BCVA OD, mean (range)</td>
<td>6/12 (HM–6/5)</td>
<td>6/12 (6/36–6/5)</td>
<td>6/18 (6/60–6/6)</td>
</tr>
<tr>
<td>BCVA OS, mean (range)</td>
<td>6/18 (HM–6/5)</td>
<td>6/12 (6/60–6/6)</td>
<td>6/24 (HM–6/5)</td>
</tr>
<tr>
<td>OD microperimetry threshold dB, median (IQR)</td>
<td>4.7 (0.6–14.0)</td>
<td>4.9 (2.9–13.6)</td>
<td>1.7 (0–4.2)</td>
</tr>
<tr>
<td>OS microperimetry threshold dB, median (IQR)</td>
<td>5.6 (0.3–10.8)</td>
<td>6.9 (1.4–10.8)</td>
<td>0.5 (0–4.0)</td>
</tr>
<tr>
<td>OD 95% fixation area degrees, median (IQR)</td>
<td>3.2 (1.5–6.7)</td>
<td>2.6 (0.9–3.9)</td>
<td>2.6 (1–5.8)</td>
</tr>
<tr>
<td>OS 95% fixation area degrees, median (IQR)</td>
<td>3.3 (1.7–8.5)</td>
<td>1.8 (0.9–3.8)</td>
<td>4.1 (18–53.5)</td>
</tr>
<tr>
<td>Distance of island edge to fovea μm OD, mean ± SD</td>
<td>+466.1 ± 962</td>
<td>+589.7 ± 862</td>
<td>−122.9 ± 513</td>
</tr>
<tr>
<td>Distance of island edge to fovea μm OS, mean ± SD</td>
<td>+321.0 ± 1132</td>
<td>+519.7 ± 783</td>
<td>−252.5 ± 849</td>
</tr>
</tbody>
</table>

IQR, interquartile range; HM, hand movements.

**FIGURE 2.** Intereye symmetry of VA and macular threshold sensitivity in patients with CHM. Intereye symmetry of BCVA (A) and mean threshold sensitivity of the central 10 degrees of the macula measured by MAIA microperimetry (B) was assessed in a cohort of 56 patients with confirmed CHM. Linear regression lines (solid lines) are shown with correlation coefficient (r) and flanked by 95% CI (two dotted lines). For reference, the line of intereye equality is shown (dashed line). The decimal VA of 2.0 represents hand movements/counting fingers acuity, which is not truly quantifiable.
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previously described. The shortest distance from the foveola to the nearest edge of the “island” of AF was measured using the HEYEX circle tool (i.e., taken as the radius of an expanding circle centered on the foveola as it first intersects an edge of the AF island). The distance from the center of the anatomical fovea to the nearest edge of degeneration (DF) was measured using the caliper tools.

A cohort of 21 of eyes (of 21 patients) underwent repeat MAIA microperimetry testing during a single visit to assess the test-retest variability of macular threshold sensitivity using Bland-Altman analysis. The follow-up function was used to ensure comparable registration. Snellen BCVA values were converted to decimal equivalents for the purpose of graphic plots. Statistical analyses were conducted with SPSS (version 22.0; IBM Software, New York, NY, USA) and GraphPad Prism (version 7.0; GraphPad Software, La Jolla, CA, USA).

A color-coded “heat-map” of the variations in macular threshold sensitivity across the whole cohort was created using QGIS (version 2.6; open source Geographic Information Systems software) from shapefiles of the combined data created using FME (Safe Software, Inc., Surrey, BC, Canada).

RESULTS

Relevant results from the microperimetry plot (Fig. 1) were recorded for analysis. We investigated binocular symmetry of visual function (BCVA and mean macular threshold sensitivity) in a cohort of 56 patients with CHM across a wide age range (mean age 38.8 years, range 12–74 years). Intereye symmetry assessed using correlation coefficient was moderate for BCVA ($r = 0.42$, $P = 0.0006$) (Fig. 2A) and very strong for threshold sensitivity of the central 10 degrees of the macula ($r = 0.95$, $P < 0.0001$, Fig. 2B). Fishers r to z transformation revealed that the correlation coefficients were not equal ($P < 0.05$). The mean threshold sensitivity differences between the eyes were $1.7 \pm 1.9$ dB.

In Figure 2B the variability appears to be greater at microperimetry thresholds below 6 dB, so a subgroup analysis of these patients was conducted (Table). The correlation coefficient of symmetry for BCVA remained similar ($r = 0.40$, $P = 0.01$) but dropped somewhat for microperimetry threshold ($r = 0.64$, $P < 0.0001$) while remaining significant. The intereye correlation of fixation stability was poor within the subgroup ($r = 0.04$, $P = 0.84$). This most likely indicates that the patients with poor vision struggle harder in performing the test, which may increase variability.

Although some intereye differences in BCVA could be present in some individuals, we speculated that the rate of decline in BCVA might remain constant with increasing age across the entire cohort. To test this hypothesis, the decline in VA with age for the right and left eyes was analyzed independently using Kaplan-Meier survival plot (SigmaPlot v13.0; SYSTAT Software, Inc., San Jose, CA, USA) with “BCVA drop below 20/20 Snellen” defined as the equivalent of “mortality” (Fig. 3A). The median survival for retaining 20/20 BCVA was 39 years for both right and left eyes; there was no statistically significant difference between the eyes ($Q^2$ for equivalence, $P > 0.999$).

Binocular decline in VA with age was also accompanied by reduction in mean threshold sensitivity of the central macula (Fig. 3B). Independent regression analysis of threshold sensitivities against age in the right and left eyes of the cohort revealed both to best fit an exponential pattern of decay: right eye half-life 13.56 (df = 51, $R^2 = 0.796$) and left eye half-life 13.60 (df = 51, $R^2 = 0.745$) within the detection range of the MAIA microperimeter (0–36 dB). Microperimetry symmetry was assessed using a Kaplan-Meier survival plot, using the definition of a difference between the eyes of greater than 1.5 dB as the equivalent of mortality (Fig. 3C). The median age at which the mean threshold sensitivity differed by more than 1.5 dB was 58 years.
microperimetry, particularly because some patients could potentially adapt by taking up eccentric fixation (Fig. 6B). We mapped the precise location of the fovea as determined by the OCT to the AF image to measure the distance from the fovea to the nearest edge of retinal degeneration (in any direction). This was used as a surrogate for the amount of foveal encroachment and correlated with macular threshold sensitivity and BCVA (Figs. 6A, 6C, respectively).

A strong linear correlation was observed between the distance from the center of the anatomical fovea to the nearest edge of degeneration ($D_j$) and mean threshold sensitivity of the central macula ($y$ intercept = 5.106; 95% confidence interval [CI] 4.292–6.040; slope = 0.0054; 95% CI 0.0054–0.0070; df = 107; $R^2 = 0.698$) (Fig. 5). Consistent with the data shown in Figure 5, the area of fixation during microperimetry testing was constant until the degeneration reached the center of the fovea, whereupon fixation stability became more variable (Fig. 6B). A similar linear decrease in BCVA was observed as the advancing front of degeneration encroached on the fovea (Fig. 6C). So, for both macular threshold sensitivity and BCVA, near normal visual functions were observed when the fovea was.

Although the data would suggest that threshold sensitivity measurements using the MAIA microperimeter could be used to monitor disease progression over time, the repeatability of microperimetry testing in our cohort of CHM patients was unknown. Microperimetry (10–2 configuration) was performed twice in the same eye at one sitting in a subcohort of 21 CHM patients (by the same tester, either JKJ or KX). The cohort was chosen to represent the full spectrum of disease stages and closely matched the original cohort of patients (mean age 35 years, range 12–70 years) as shown in the Table. Bland-Altman repeatability analysis suggested that a difference in mean threshold sensitivity of 1.45 dB has a 95% chance of being within test-retest variability in CHM (Fig. 4A). This is in keeping with previous reports. Furthermore, the repeatability of MAIA microperimetry was further assessed in a subset of 11 patients (mean age 38 years, range 27–55 years) who underwent repeat testing on different days and at different times of day. Two tests were undertaken on two separate days with a maximum gap of 40 days apart, median difference between tests was 28 days and the range was 20 to 38 days apart. All these tests were undertaken at the same time of day by a single examiner (JJ). The coefficient of repeatability (CR) was 1.05 (95% limits of agreement +1.05 to –1.95) (Fig. 4B). The same subset of patients also underwent testing at different times of day, with one test being conducted in the morning clinic session and the second test being conducted in the afternoon clinic session with a median test interval of 241 minutes (range 168 minutes to 387 minutes) between tests. The CR was 1.67 (95% limits of agreement +1.05 to –1.95) (Fig. 4C). From the CR in the three situations, they do not appear to be clinically different. A potential confounding factor in the repeatability of microperimetry testing was fixation stability. This was generally well maintained, but began to drop once the mean threshold sensitivity fell below 6 dB (Fig. 5).

The ability of the patient to “fixate” on a visual target, such as a small letter on a distant VA chart (during Early Treatment Diabetic Retinopathy Study BCVA testing) or the central fixation target during microperimetry, could have a significant impact on the reliability of the test. Fixation is highly dependent on foveal integrity, which could become affected in CHM as the centripetal degeneration advances. However, it is unclear how close the edge of degeneration needs to be to the center of the fovea to cause a deleterious effect on BCVA or microperimetry, particularly because some patients could.

**FIGURE 4.** Repeatability of MAIA microperimetry in CHM patients. (A) MAIA 10–2 microperimetry was performed twice in the same eye (right eye) at the same sitting in a subcohort of 21 CHM patients over a spectrum of disease severity. Bland-Altman repeatability analysis showed a CR of 1.45 (95% limits of agreement +1.24 to –1.62). (B) Further repeatability analysis on a subset of 11 patients over 2 days at the same time of day, show a CR of 1.05 (95% limits of agreement –0.62 to –1.22). (C) Repeatability analysis on the same subset of 11 CHM patients who undertook microperimetry in the morning and afternoon of the same day, showing a CR of 1.67 (95% limits of agreement +1.05 to –1.95).

**FIGURE 5.** The area covered by 95% of the fixation points during the test time was plotted against mean threshold sensitivity. Fixation stability appeared to deteriorate once the mean threshold sensitivity falls below 6 dB.
Although the mean threshold sensitivity of the central 10° of the macula appeared to follow exponential decay with age (Fig. 3B), zonal analyses revealed asymmetry in the rate of decline between the temporal and nasal aspect of the central macula. By creating composite threshold sensitivity heat-maps of the summed responses across the cohort (112 eyes of 56 patients), preservation of sensitivity could be seen at the parafoveal regions, while sensitivity declined faster nasal to the fovea than temporal (Fig. 7A). The means of the summed threshold responses of the central, nasal, and temporal macula were compared using ANOVA testing with Bonferroni correction (Fig. 7B). The intereye differences were not statistically significant ($P = 0.65$); however, the nasal and temporal retinal sensitivity was lower than central sensitivity ($P = 0.02$). The nasal sensitivity was lower than temporal sensitivity ($P = 0.02$).

**Discussion**

Choroideremia is an X-linked monogenic retinal dystrophy with 100% penetrance (mostly due to nonsense mutations) and, as such, would be expected to affect both eyes to a similar degree in affected males. Binocular symmetry of disease progression and visual function is an important assumption that forms the basis of clinical trial design. For instance, gene therapy trials using adeno-associated viral vector to deliver a normal copy of the CHM gene to the diseased retina involves subretinal delivery of vector to one eye and the fellow eye acting as an internal control.

Our data demonstrated MAIA microperimetry to be a repeatable test in CHM. Our repeatability coefficient (1.45 dB) is similar to that previously reported in CHM and lower than that reported in AMD. The CR holds under different conditions (i.e., different days or times of day), although these additional factors were tested in a subset of patients with better vision than the general cohort. The superior reliability of microperimetry in CHM may be due to foveal sparing degeneration enabling adequate fixation in most patients, tests being conducted by only two observers, or the fact that our cohort was relatively experienced in performing microperimetry due to the test being part of regular follow-up to monitor disease progression. The mean threshold sensitivity of the 10–2 standard macular grid appeared to show a high degree of intereye symmetry and therefore is valuable as an outcome measure for comparing treated and control eyes in clinical trials. BCVA, on the other hand showed moderate intereye asymmetry in later stages of the disease. The greater intereye differences seen in advanced CHM (subgroup analysis of low-threshold patients) is likely to arise from differences in foveal involvement as the centripetal degeneration encroaches on the fovea earlier in one eye than the other, leading to greater loss of foveal cones. It is therefore possible for the area of residual functional retina (as seen by AF imaging) in both eyes to be relatively symmetrical, while the BCVA is significantly different due to differences in the extent of foveal degeneration. Microperimetry measurements, in contrast, would be less affected by intereye differences in foveal degeneration as the macular thresholds represent the mean of the entire central 20 degrees of field, except in end-stage disease when fixation instability becomes significant in one eye but not the other.

Other possible contributing factors to intereye asymmetry of BCVA or macular sensitivity may include unilateral cophathologies, such as cataract, degenerative foveoschisis, unknown amblyopia, epiretinal membrane, or previous choroidal neovascularization, which could occasionally occur in association with choroideremia; however, none of these were included in this series.
In quantifying the vision-critical minimum distance between the fovea and the edge of autofluorescent islands as well as the critical period during which BCVA decline occurs, the data demonstrate a key structure-function correlation in CHM. \( D_f \) would appear to be a crucial prognostic factor to discuss with CHM patients, as it would give an indication as to how much time might remain before foveal involvement occurs, which would have a major impact on quality of life. Once the edge of degeneration reached 500 \( \mu m \) or closer to the foveal center, BCVA appeared to undergo relatively rapid decline. A 20/20 letter will measure 24.8 \( \mu m \) on the retina. The decline in VA from 500 \( \mu m \) onward is therefore unlikely to be due to reduction in visual tracking ability but rather a direct result of cone photoreceptor loss. Our visual function results are in keeping with those previously reported in CHM and indicate a slower rate of decline in VA with age than those reported in X-linked rod-cone dystrophies. This would imply that a larger therapeutic window exists for intervention in CHM before severe vision loss.

Central AF island area shrinkage in CHM has previously been reported to have a half-life of approximately 9 years. Cone function in retinitis pigmentosa has been reported to have a half-life of 7 years as defined by amplitude of ERGs. We found the half-life of macular threshold sensitivity decline to be approximately 13.6 years for both eyes. The discrepancy may be explained by the sensitivity range of the microperimeter (0–36 dB), which means that thresholds <0 dB (which exist due to the logarithmic unit) could not be detected, therefore skewing the correlation curve upward. In addition, microperimetry tested the central 20 degrees of field loss and therefore covered a smaller area than the 55-degree AF images originally reported, indicating central preservation until late into the disease.

In terms of the pattern of retinal function loss, the nasal retinal sensitivity appeared to decline earlier than temporal retina, which mirrors a similar pattern of AF island shrinkage reported previously. We hypothesize that this asymmetry of retinal degeneration on either side of the fovea may be related...
to differences in nasal and temporal rod density, melanin concentration, or choroidal vascular supply. Interestingly, the choroidal blood supply is most dense centrally, which may provide a protective effect on the foveal cones against early degeneration, providing potential support for the choroidal blood supply theory.

Fixation stability is dependent on foveal cone and possibly Müller cell function, and is an important determinant of reading speed due to its role in visual tracking. Our microperimetry data would suggest that fixation stability in CHM is well preserved until late into the disease state, further supporting the importance of early intervention to maximize preservation of visual function. A lag between RPE loss and overlying photoreceptor loss might explain why visually active points (points with a sensitivity >0 dB) were occasionally detected on microperimetry in regions with no measurable RPE by AF imaging.

The results support the validity of both BCVA and macular thresholds sensitivity as valuable outcome measures for clinical trials to treat CHM. Both visual functional modalities demonstrated binocular symmetry, particularly in early stages of the disease, lending themselves to randomization and the use of the contralateral eye as a control in clinical trials. BCVA has a direct impact on the quality of daily living and is therefore an important endpoint in clinical trials, but fluctuations in BCVA in advanced CHM need to be interpreted with respect to the distance between the fovea and the edge of the residual AF island. In contrast, microperimetry threshold sensitivity represents a more global response from the central macula and yet could also provide “point-to-point” information within the region tested.

Acknowledgments

The authors thank Jon Brett, medical photographer in the Eye Research Group Oxford for conducting patient OCT and AF imaging.

Supported by the National Institute for Health Research (NIHR) Oxford Biomedical Research Centre based at Oxford University Hospitals NHS Trust and University of Oxford, The Health Innovation Challenge Fund, and Fight for Sight. The views expressed are those of the authors and not necessarily those of the NHS, the NIHR, or the Department of Health. The sponsor and funding organization had no role in the design or conduct of this research.

Disclosure: J.K. Jolly, National Health Service (E); K. Xue, National Health Service (E); T.L. Edwards, National Health Service (E); M. Groppe, National Health Service (E); R.E. MacLaren, National Health Service (E), NightstaRx Ltd (S)

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