Published in final edited form as:

Ophthalmic Genet. 2018 August ; 39(4): 437–442. doi:10.1080/13816810.2018.1466340.

Diurnal variations of foveoschisis by optical coherence tomography in patients with *RS1* X-linked retinoschisis

Maria Fernanda Abalem^{1,2}, David C. Musch^{1,3}, David G. Birch⁴, Mark E. Pennesi⁵, John R. Heckenlively¹, and Thiran Jayasundera¹

¹Department of Ophthalmology and Visual Sciences, Kellogg Eye Center, University of Michigan Medical School, Ann Arbor, Michigan, United States

²Department of Ophthalmology and Otolaryngology, University of Sao Paulo Medical School, Sao Paulo, Sao Paulo, Brazil

³Department of Epidemiology, University of Michigan School of Public Health, Ann Arbor, Michigan, United States

⁴Retina Foundation of the Southwest, Dallas, Texas, United States

⁵Oregon Health & Science University, Casey Eye Institute, Portland, Oregon, United States

Abstract

Background: To evaluate diurnal variations in macular schisis cavities in patients with X-linked juvenile retinoschisis (XLRS) with pathogenic variants in the *RS1* gene using spectral domain optical coherence tomography (SD-OCT).

Methods: Three consecutive patients with a clinical diagnosis of XLRS and pathogenic variants in the *RS1*, treated with carbonic anhydrase inhibitors (CAIs).

Observational procedures: SD-OCT scans of the macula were acquired at 9am, 1 pm and 4pm within 24 hours.

Results: All patients demonstrated increased measures of central foveal thickness in the morning with gradual decrease through the day (9 to 43%). Major changes were observed between 9am and 1pm in the central foveal thickness.

Conclusion: The central foveal thickness varies during daytime hours in patients with XLRS. This finding may explain the inconsistent and heterogeneous responses to treatment with carbonic anhydrase inhibitors and necessitate standardization of measurement times in treatment trials for XLRS as well as in the routine ophthalmic evaluation of these patients.

Keywords

retinoschisis; inherited retinal degenerations; macular edema; optical coherence tomography; x-linked; genetics

Corresponding author and address for reprints: Thiran Jayasundera, MD, 1000 Wall Street, Ann Arbor, MI, 48105, thiran@med.umich.edu, Phone: (734) 232-8080; Fax: (734) 764 390.

Disclosure Statement: The authors have nothing to disclose.

This manuscript has not been published elsewhere and has not been submitted simultaneously for publication elsewhere.

Introduction

X-linked retinoschisis (XLRS) is an early onset and slowly progressive degeneration of the retina and macula,¹ caused by pathogenic variants in the *RS1* (retinoschisin) gene located in the Xp22.1 chromosome.² The *RS1* gene encodes the retinoschisin protein that is involved in maintaining the retinal structure, by binding the surface of photoreceptors and bipolar cells.^{3,4} Mutations in the *RS1* gene lead to a splitting of the neural retina and dysfunction in the ON- and OFF-pathways, that translate into a reduced b/a-ratio on the full field electroretinogram (ffERG) and reduced visual acuity in the first decade of life.⁵ Clinically, patients with XLRS present with schisis of the macula in a spoke wheel pattern observed mostly in the nuclear layers, but also in the plexiform, ganglion cells and nerve fiber layers.^{6–9} In some patients, schisis cavities extend to the peripheral retina and complications such as vascular sheathing, vitreous veils, vitreous haemorrhage, retinochoroidal neovascularization and retinal detachment may develop.¹⁰ Over time, usually during the 4th decade of life, the schisis cavities collapse and progressive macular atrophy ensues with a decline in visual acuity.^{10,11}

Currently there is no approved treatment for XLRS. Nevertheless, interventions to reduce the schisis cavities are considered beneficial in delaying the development of macular atrophy and deterioration in visual acuity.^{12,13} Several small and nonrandomized studies have reported the use of oral and topical carbonic anhydrase inhibitors (CAIs) in the management of schisis cavities in patients with XLRS. Documentation of an efficacious response to these agents is lacking in consistency.^{14,15} While in some patients there was a reduction of macular cavities;^{13,16} in others, there was no improvement and even an increase in the macular thickness.^{15,17,18} Spontaneous resolution has also been described in untreated eyes.¹⁹ Jeffrey et al evaluated the inter-visit variability of outcome measures including the retinal thickness in patients with XLRS and showed changes ranging from a 22% decrease to a 28% increase in the central retinal thickness by optical coherence tomography (OCT).²⁰ Similarly, Apushkin et al demonstrated an inter-visit change of 19.6% of the foveal thickness by OCT in four untreated patients with XLRS.¹³

In addition to inter-visit variability,²¹ diurnal variations of retinal thickness may also occur. Studies in patients with cystoid macular edema (CME) secondary to diabetic retinopathy (DR)^{22–25} and to central retina vein occlusion (CRVO)^{26,27} have demonstrated a mild increase of central macular thickness in the morning, with gradual reduction during the remaining hours of the day.^{26,27,22–24} There is a need to better understand the degree of diurnal variation of schisis cavities in patients with XLRS, as the efficacy of novel treatments such as *RS1* gene therapy includes macular thickness and cyst volume by OCT as an outcome measures, and clinicians use OCT imaging to evaluate response to CAI therapy as standard of care. The aim of this study was to evaluate the diurnal variation of foveoschisis assessed by OCT in patients with a clinical diagnosis of XLRS and a documented pathogenic variant in the *RS1* gene.

Methods

This case series was performed at the Kellogg Eye Center, University of Michigan. The study was approved by the Michigan Institutional Review Board and conducted according to the Declaration of Helsinki. Informed consent was obtained from patients older than 18 years or from the parent or legally authorized representative of patients younger than 18 years. Informed assent was also obtained from children older than 9 years.

We included three patients 8 years of age or older with both a clinical and genetic molecular diagnosis of XLRS who presented consecutively for clinical evaluation at the Kellogg Eye Center. The clinical diagnosis was established by the presence of foveoschisis with or without peripheral retinoschisis. Genetic molecular diagnosis was established by the detection of at least one variant in the *RS1* gene that was classified by the genetic testing laboratory and *in silico* tools as either pathogenic or likely pathogenic. Patients were excluded if they had intraocular surgery within the past 3 months; a diagnosis of any other retinal disease besides XLRS; prior history of retinal detachment; or any ocular opacity that would result in poor image quality. Patients without foveoschisis at the time of the exam and female carriers were not included.

All patients underwent a comprehensive ocular examination, including visual acuity on Snellen chart, biomicroscopy of anterior and posterior segments, intraocular pressure, and Spectral-Domain Optical Coherence Tomography (SD-OCT) (Spectralis; Heidelberg Engineering, Heidelberg, Germany).

SD-OCT images were acquired at 9am, 1pm and 4pm using the automated eye alignment eye-tracking software (TruTrack; Heidelberg Engineering) to obtain 25 high-speed horizontal line scans of the macula of both eyes within the central 20° centered in the fovea. The Early Treatment Diabetic Retinopathy Study (ETDRS) grid was selected to obtain numeric averages of retinal thickness. This grid consists of 3 rings of 1 mm (inner), 3 mm (intermediate), and 6 mm (outer) diameter centered at the fovea. The measures obtained within the inner 1 mm circle are defined as central foveal thickness, and the intermediate and outer rings are divided into four fields: superior, nasal, inferior, and temporal. The macular thickness was obtained in each of the nine fields of the ETDRS grid. The thicknesses of inferior, superior, temporal and nasal fields were obtained by calculating the average between the respective fields at the intermediate and outer rings.

Given the small sample size, descriptive data is presented, and variations in retinal thickness were calculated and displayed as absolute and relative values (%).

Results

We included 3 unrelated patients with XLRS who had documented pathogenic variants in the *RS1* with foveoschisis. Demographic, clinical and genetic data are shown in Table 1. Two patients were using dorzolamide three times a day in both eyes and one patient was using acetazolamide 250 mg daily. They did not use these medications on the day of the exam.

All patients showed changes in all macular thickness fields during the day, more prominent in the central fovea (9% to 43%). The major variations (reduction of schisis) were observed between 9am and 1pm, and minor reductions were observed between 1pm and 4 pm. The changes were observed in both eyes and varied among patients. Patient 1, who was on topical CAI, showed significant and symmetrical changes; patient 2, who was on oral acetazolamide, showed small and symmetrical changes; and patient 3, who was on topical CAI, showed asymmetrical variation. Table 2 shows the percentage variation between time points of the central foveal thickness of the three patients. Figure 1 shows the SD-OCT images of the right eye of patient 1 at 9 am, 1 pm and 4 pm. There were also differences in macular thickness in the inferior, superior, temporal and nasal fields. Due to the difficulty of performing SD-OCT layer-by-layer segmentation in this disease, subjective SD-OCT assessment indicated that the outer nuclear layer variation in thickness impacted more on the overall thickness variation compared to the other retinal layers. The central foveal thickness of both eyes at the three time points are displayed in Figure 2.

Discussion

In this study, we evaluated the diurnal variation of foveoschisis by SD-OCT measurements of the macula in patients who have XLRS accompanied by pathogenic variants in the *RS1*. We observed diurnal variation in foveoschisis with greater central foveal thickness in the morning and a sharp decrease at midday followed by a milder decrease by late afternoon. To our knowledge, this degree of diurnal variation in the macular thickness of patients with XLRS has not been reported. Diurnal variation as we document would impact the manner with which treatment outcomes are assessed for this disease, which currently entails monitoring response to CAI treatment based on OCT evaluation. This pertains not only to patient-specific monitoring, but also and importantly to therapeutic trials for this disease.

Several studies have described diurnal variations in patients with CME secondary to DR and CRVO, with a similar pattern as observed in this study. In 1982, Sternberg et al described three patients with macular edema secondary to DR and CRVO who experienced variations in visual acuity within the same day, suggesting cyclic changes in macular edema during the day.²⁵ Later, with the advent of OCT, studies have demonstrated variations in macular thickness in DME and CME secondary to CRVO with the greatest change observed between 8 and 11 am.^{22–24,26,27} In CME, several hypotheses have been proposed to explain this pattern, such as gravitational shift from a recumbent position during the night time to an upright position during the day; arterial pressure variations; and circadian changes in the secretion of cortisol that ultimately would interfere with vascular integrity.^{24,27} Given that the pathogenesis of CME in patients with DR and CRVO essentially involves dysfunction of the inner blood retinal barrier and that most patients commonly have other systemic vascular diseases, such as systemic hypertension, it is possible that vascular mechanisms, for example, variations in venous and arterial blood pressure, play a role in these cases. In patients with XLRS, however, mechanisms based on vascular changes seem to be less likely. Although there is evidence of vascular abnormalities in XLRS,²⁸ macular leakage on fluorescein angiography is rare.¹² In addition, systemic vascular diseases are uncommon in children and so are variations in arterial blood pressure. If gravitational shifts impact the retinal thickness through the day, it would also be expected that the accumulation of fluid

would disperse inferiorly through the course of the day. In our study, we did not observe a substantial difference between superior and inferior macular thicknesses and it seems less likely that gravitational shifts account for the large diurnal variation of foveoschisis in patients with XLRS.

Although diurnal variations in the central macular thickness in CME and foveoschisis followed a similar pattern, the changes observed in this study were greater (9–43%) than the change observed in patients with CME (6–22%).^{22–24,26,27} We hypothesize that mechanisms underlying the retinoschisin function may be involved in diurnal variations in XLRS. Besides playing a role in the maintenance of the retinal architecture,³ retinoschisin is thought to regulate the fluid balance between intracellular and extracellular spaces at the photoreceptor and bipolar cell layers by binding to Na/K ATPase, also known as the sodium pump, present in these cells.^{12,29,30} Therefore, it is possible that modulation of the activity of the Na/K ATPase may further interfere with the fluid balance at the photoreceptors-bipolar cells synapses during the 24 hour cycle. In neurons, there is a diurnal rhythm of the Na/K ATPase activity, when the activity is higher during the day.^{31,32} In addition, insulin, aldosterone, glucocorticosteroids, and epinephrine, hormones known to be increased during the day, stimulate the sodium pump in several tissues.³³ Thus, it is possible that the Na/K ATPase activity in the photoreceptors, bipolar cells and even in the retinal pigment epithelium may be enhanced during the day, contributing to fluid absorption, and reduction of macular thickness over the day.

It is also noteworthy that the expression and secretion of retinoschisin are under circadian control. In normal conditions, the secretion of retinoschisin has a peak at night and trough during the day.^{34–36} It is possible that patients with pathogenic variants in the *RS1* may have an impaired circadian rhythm. Hence, lower levels of retinoschisin at night would decrease fluid absorption resulting in more fluid in the morning, which was the pattern observed in this study.

Although this study did not evaluate the variability of macular thickness between visits, it is possible that the inter-visit variability previously reported may be inaccurate, because these studies may have measured the macular thickness at different times at each visit. The same rationale applies to studies that evaluated the use of CAIs and may explain the heterogeneous responses to CAI therapy. One limitation of our study is the small sample size. A larger study with consistent and exact imaging times is needed to provide a robust estimate of diurnal variation of foveal schisis in patients with XLRS. Furthermore, larger studies are warranted to investigate factors associated with the variability of macular thickness among patients and between eyes. This study is unable to evaluate the contribution of CAIs on the diurnal variation of macular schisis. However, withholding CAIs on the day of testing still led to reduction of macular thickness during the course of the day. Functional tests such as visual acuity and microperimetry were not performed at all time points and therefore, the impact of schisis variations on central visual function remains unclear. Therapeutic studies assessing efficacy may need to measure visual function at consistent and exact times as well. However, given that the thickness of photoreceptors outer segment correlates better with macular function than total retinal thickness, it is possible that diurnal variations in foveal schisis may not affect the visual function.⁹

In conclusion, this study demonstrated that macular thickness assessed by SD-OCT shows significant diurnal variations with greater central foveal thickness in the morning in patients with pathogenic variants in *RS1*. Clinicians need to be aware of this diurnal variation in order to assess the response that each patient has to CAI or any future therapeutic agent. The optimal clinical care would be to perform baseline OCT imaging throughout one day and then evaluate the effect of therapy in follow up visits by comparing to images acquired at baseline testing at a similar time of the day, which is comparable to intraocular pressure monitoring in glaucoma. Future and current therapeutic trials need to be aware of this diurnal variation and assess variations in macular thickness and central visual function at consistent times in follow up study visits.

Acknowledgements:

We would like to thank Ms. Kari Branham, Ms. Dana Schlegel and Cynthia Qian, MD for their valuable insights.

Funding: This research was supported by the Foundation Fighting Blindness grant CF-CL-0616-0698-UMICH and by the National Institute of Health grant K23EY026985.

References

1. George ND, Yates JR, Bradshaw K, Moore AT. Infantile presentation of X linked retinoschisis. *Br J Ophthalmol* 1995;79(7):653–657. [PubMed: 7662629]
2. Sauer CG, Gehrig A, Warneke-Wittstock R, et al. Positional cloning of the gene associated with X-linked juvenile retinoschisis. *Nat Genet* 1997;17(2):164–170. [PubMed: 9326935]
3. Molday LL, Hicks D, Sauer CG, Weber BH, Molday RS. Expression of X-linked retinoschisis protein RS1 in photoreceptor and bipolar cells. *Invest Ophthalmol Vis Sci* 2001;42(3):816–825. [PubMed: 11222545]
4. Wu WW, Wong JP, Kast J, Molday RS. RS1, a discoidin domain-containing retinal cell adhesion protein associated with X-linked retinoschisis, exists as a novel disulfide-linked octamer. *J Biol Chem* 2005;280(11):10721–10730. [PubMed: 15644328]
5. Khan NW, Jamison JA, Kemp JA, Sieving PA. Analysis of photoreceptor function and inner retinal activity in juvenile X-linked retinoschisis. *Vision Res* 2001;41(28):3931–3942. [PubMed: 11738458]
6. Apushkin MA, Fishman GA, Janowicz MJ. Correlation of optical coherence tomography findings with visual acuity and macular lesions in patients with X-linked retinoschisis. *Ophthalmology* 2005;112(3):495–501. [PubMed: 15745780]
7. Gregori NZ, Berrocal AM, Gregori G, et al. Macular spectral-domain optical coherence tomography in patients with X linked retinoschisis. *Br J Ophthalmol* 2009;93(3):373–378. [PubMed: 19019942]
8. Yu J, Ni Y, Keane PA, Jiang C, Wang W, Xu G. Foveomacular schisis in juvenile X-linked retinoschisis: an optical coherence tomography study. *Am J Ophthalmol* 2010;149(6):973–978.e972. [PubMed: 20430364]
9. Bennett LD, Wang YZ, Klein M, Pennesi ME, Jayasundera T, Birch DG. Structure/Psychophysical Relationships in X-Linked Retinoschisis. *Invest Ophthalmol Vis Sci* 2016;57(2):332–337. [PubMed: 26830370]
10. Apushkin MA, Fishman GA, Rajagopalan AS. Fundus findings and longitudinal study of visual acuity loss in patients with X-linked retinoschisis. *Retina* 2005;25(5):612–618. [PubMed: 16077359]
11. Menke MN, Feke GT, Hirose T. Effect of aging on macular features of X-linked retinoschisis assessed with optical coherence tomography. *Retina* 2011;31(6):1186–1192. [PubMed: 21386765]
12. Molday RS, Kellner U, Weber BH. X-linked juvenile retinoschisis: clinical diagnosis, genetic analysis, and molecular mechanisms. *Prog Retin Eye Res* 2012;31(3):195–212. [PubMed: 22245536]

13. Apushkin MA, Fishman GA. Use of dorzolamide for patients with X-linked retinoschisis. *Retina* 2006;26(7):741–745. [PubMed: 16963845]
14. Gurbaxani A, Wei M, Succar T, McCluskey PJ, Jamieson RV, Grigg JR. Acetazolamide in retinoschisis: a prospective study. *Ophthalmology* 2014;121(3):802–803.e803. [PubMed: 24309554]
15. Walia S, Fishman GA, Molday RS, et al. Relation of response to treatment with dorzolamide in X-linked retinoschisis to the mechanism of functional loss in retinoschisin. *Am J Ophthalmol* 2009;147(1):111–115.e111. [PubMed: 18834580]
16. Verbakel SK, van de Ven JP, Le Blanc LM, et al. Carbonic Anhydrase Inhibitors for the Treatment of Cystic Macular Lesions in Children With X-Linked Juvenile Retinoschisis. *Invest Ophthalmol Vis Sci* 2016;57(13):5143–5147. [PubMed: 27699410]
17. Grover S, Apushkin MA, Fishman GA. Topical dorzolamide for the treatment of cystoid macular edema in patients with retinitis pigmentosa. *Am J Ophthalmol* 2006;141(5):850–858. [PubMed: 16546110]
18. Andreuzzi P, Fishman GA, Anderson RJ. USE OF A CARBONIC ANHYDRASE INHIBITOR IN X-LINKED RETINOSCHISIS: Effect on Cystic-Appearing Macular Lesions and Visual Acuity. *Retina* 2017;37(8):1555–1561. [PubMed: 27828908]
19. Jo YJ, Kim KN, Kim JY. Spontaneous resolution of foveal cysts associated with X-linked retinoschisis as observed by optical coherence tomography. *Can J Ophthalmol* 2010;45(4):414–415. [PubMed: 20648079]
20. Jeffrey BG, Cukras CA, Vitale S, Turriff A, Bowles K, Sieving PA. Test-Retest Intervisit Variability of Functional and Structural Parameters in X-Linked Retinoschisis. *Transl Vis Sci Technol* 2014;3(5):5.
21. Browning DJ, Fraser CM, Propst BW. The variation in optical coherence tomography-measured macular thickness in diabetic eyes without clinical macular edema. *Am J Ophthalmol* 2008;145(5):889–893. [PubMed: 18329622]
22. Frank RN, Schulz L, Abe K, Iezzi R. Temporal variation in diabetic macular edema measured by optical coherence tomography. *Ophthalmology* 2004;111(2):211–217. [PubMed: 15019364]
23. Larsen M, Wang M, Sander B. Overnight thickness variation in diabetic macular edema. *Invest Ophthalmol Vis Sci* 2005;46(7):2313–2316. [PubMed: 15980216]
24. Polito A, Polini G, Chiodini RG, Isola M, Soldano F, Bandello F. Effect of posture on the diurnal variation in clinically significant diabetic macular edema. *Invest Ophthalmol Vis Sci* 2007;48(7):3318–3323. [PubMed: 17591904]
25. Sternberg P Jr., Fitzke F, Finkelstein D. Cyclic macular edema. *Am J Ophthalmol* 1982;94(5):664–669. [PubMed: 7148947]
26. Gupta B, Grewal J, Adewoyin T, Pelosini L, Williamson TH. Diurnal variation of macular oedema in CRVO: prospective study. *Graefes Arch Clin Exp Ophthalmol* 2009;247(5):593–596. [PubMed: 19052771]
27. Paques M, Massin P, Sahel JA, et al. Circadian fluctuations of macular edema in patients with morning vision blurring: correlation with arterial pressure and effect of light deprivation. *Invest Ophthalmol Vis Sci* 2005;46(12):4707–4711. [PubMed: 16303968]
28. Rao P, Robinson J, Yonekawa Y, et al. WIDE-FIELD IMAGING OF NONEXUDATIVE AND EXUDATIVE CONGENITAL X-LINKED RETINOSCHISIS. *Retina* 2016;36(6):1093–1100. [PubMed: 26655612]
29. Molday LL, Wu WW, Molday RS. Retinoschisin (RS1), the protein encoded by the X-linked retinoschisis gene, is anchored to the surface of retinal photoreceptor and bipolar cells through its interactions with a Na/K ATPase-SARM1 complex. *J Biol Chem* 2007;282(45):32792–32801. [PubMed: 17804407]
30. Friedrich U, Stohr H, Hilfinger D, et al. The Na/K-ATPase is obligatory for membrane anchorage of retinoschisin, the protein involved in the pathogenesis of X-linked juvenile retinoschisis. *Hum Mol Genet* 2011;20(6):1132–1142. [PubMed: 21196491]
31. Wang HY, Huang RC. Diurnal modulation of the Na⁺/K⁺-ATPase and spontaneous firing in the rat retinorecipient clock neurons. *J Neurophysiol* 2004;92(4):2295–2301. [PubMed: 15381747]

32. Ko GY, Shi L, Ko ML. Circadian regulation of ion channels and their functions. *J Neurochem* 2009;110(4):1150–1169. [PubMed: 19549279]
33. Therien AG, Blostein R. Mechanisms of sodium pump regulation. *Am J Physiol Cell Physiol* 2000;279(3):C541–566. [PubMed: 10942705]
34. Ko ML, Liu Y, Shi L, Trump D, Ko GY. Circadian regulation of retinoschisin in the chick retina. *Invest Ophthalmol Vis Sci* 2008;49(4):1615–1621. [PubMed: 18385082]
35. Shi L, Jian K, Ko ML, Trump D, Ko GY. Retinoschisin, a new binding partner for L-type voltage-gated calcium channels in the retina. *J Biol Chem* 2009;284(6):3966–3975. [PubMed: 19074145]
36. Shi L, Ko ML, Ko GY. Retinoschisin Facilitates the Function of L-Type Voltage-Gated Calcium Channels. *Front Cell Neurosci* 2017;11:232. [PubMed: 28848397]
37. Vincent A, Robson AG, Neveu MM, et al. A phenotype-genotype correlation study of X-linked retinoschisis. *Ophthalmology* 2013;120(7):1454–1464. [PubMed: 23453514]

Figure 1. SD-OCT images of the right eye of patient #1 at 9 am (top), 1 pm (middle) and 4 pm (bottom). Note the presence of numerous and coalescent schisis cavities mostly in the fovea at 9am with gradual decrease in volume through the day.

Figure 2.
Central foveal thickness of both eyes of the three patients at 9am, 1pm and 4pm.

Table 1.

Demographic, clinical and genetic data of the three patients.

Variable	Patient # 1	Patient # 2	Patient # 3
Age (years)	8	14	12
<i>RS1</i> pathogenic variant	c.286T>C (p.W96R)	Deletion of exon 2	c. 208 G>A (p.G70S)
CAI treatment	Dorzolamide 1%	Acetazolamide 250mg	Dorzolamide 1%
Peripheral retinoschisis	Inferior	No	No
BCVA OD	20/40	20/60	20/125
BCVA OS	20/50	20/60	20/30
IOP OD (mmHg)	17	15	17
IOP OS (mmHg)	19	14	16

CAI: carbonic anhydrase inhibitors

BCVA: best corrected visual acuity

OD: right eye

OS: left eye

Table 2.

Relative variation (%) between time points of the central foveal thickness of the three patients.

Central Foveal thickness (μm)	9:00 AM		1 PM (%)		4 PM (%)	
	OD	OS	OD	OS	OD	OS
Patient #1	521	529	−36%	−35%	−42%	−43%
Patient #2	367	383	−10%	−7%	−13%	−9%
Patient #3	542	476	−13%	−28%	−17%	−31%

% : percent change from 9 AM measure