

LONDON
SCHOOL of
HYGIENE
& TROPICAL
MEDICINE



Gichuhi, S; Macharia, E; Kabiru, J; Zindamoyen, AM; Rono, H; Ol-
lando, E; Wanyonyi, L; Wachira, J; Munene, R; Onyuma, T; Sagoo,
MS; Weiss, HA; Burton, MJ (2015) Clinical Presentation of Ocular
Surface Squamous Neoplasia in Kenya. *JAMA ophthalmology*, 133
(11). pp. 1305-13. ISSN 2168-6165 DOI: <https://doi.org/10.1001/jamaophthalmol.2015.3335>

Downloaded from: <http://researchonline.lshtm.ac.uk/2305215/>

DOI: [10.1001/jamaophthalmol.2015.3335](https://doi.org/10.1001/jamaophthalmol.2015.3335)

Usage Guidelines

Please refer to usage guidelines at <http://researchonline.lshtm.ac.uk/policies.html> or alternatively contact researchonline@lshtm.ac.uk.

Available under license: <http://creativecommons.org/licenses/by-nc-nd/2.5/>

1 **Clinical Presentation of Ocular Surface Squamous Neoplasia in**
2 **Kenya**

3
4 **Authors:**

5 Stephen Gichuhi, M.Med^{1,2}, Ephantus Macharia, HND³, Joy Kabiru, M.Med³, Alain M'bongo
6 Zindamoyen, M.Med³, Hilary Rono, M.Med⁴, Ernest Ollando, M.Med⁵, Leonard Wanyonyi,
7 HND⁵, Joseph Wachira, M.Med⁶, Rhoda Munene, M.Med⁶, Timothy Onyuma, M.Med⁷,
8 Mandeep S. Sagoo, FRCOphth^{8,9,10}, Helen A. Weiss, PhD¹ and Matthew J. Burton PhD^{1,9}

9
10 **Email contacts**

11 Stephen Gichuhi, stephen.gichuhi@lshtm.ac.uk
12 Ephantus Macharia, machariaew@yahoo.co.uk
13 Joy Kabiru, joykabiru@yahoo.com
14 Alain M'bongo Zindamoyen, zindamoyen@yahoo.com
15 Hilary Rono, hkrono@yahoo.com
16 Ernest Ollando, eollando@yahoo.com
17 Leonard Wanyonyi, leosab09@yahoo.com
18 Joseph Wachira, drjwachira@gmail.com
19 Rhoda Munene, drmmunene@gmail.com
20 Timothy Onyuma, tonyuma@mpshahhosp.org
21 Mandeep S. Sagoo, mandeep.sagoo@moorfields.nhs.uk
22 Helen A. Weiss, helen.weiss@lshtm.ac.uk
23 Matthew J. Burton, matthew.burton@lshtm.ac.uk

24
25 **Affiliations:**

26 ¹ London School of Hygiene and Tropical Medicine, Keppel Street, London, WC1E 7HT, UK
27 ² Department of Ophthalmology, University of Nairobi, P.O Box 19676-00202, Nairobi, Kenya
28 ³ PCEA Kikuyu Eye Unit, PO Box 45, Kikuyu, Kenya
29 ⁴ Kitale District Hospital, PO Box 98-30200, Kitale, Kenya
30 ⁵ Sabatia Eye Hospital, PO Box 214-50311, Wodanga, Kenya
31 ⁶ Kenyatta National Hospital, PO Box 20723-00202, Nairobi, Kenya
32 ⁷ MP Shah Hospital, Department of Pathology, PO Box 14497-00800, Nairobi, Kenya
33 ⁸ UCL Institute of Ophthalmology, 11-43 Bath Street, London EC1V 9EL, UK
34 ⁹ Moorfields Eye Hospital, 162 City Road, London, EC1V 2PD, UK
35 ¹⁰ St. Bartholomew's Hospital, W Smithfield, London EC1A 7BE, London, UK

36
37
38 **Corresponding Author:**

39 Stephen Gichuhi
40 International Center for Eye Health
41 London School of Hygiene & Tropical Medicine
42 Keppel Street, London WC1E 7HT
43 United Kingdom
44 Tel. +44 (20) 7636 8636 ext. 8257
45 stephen.gichuhi@lshtm.ac.uk

46
47
48 **Word count:** Abstract 350; Text 2991

49 **Abstract**

50 IMPORTANCE

51 There is a trend towards treating conjunctival lesions suspected to be ocular surface
52 squamous neoplasia (OSSN) based on the clinical impression.

53

54 OBJECTIVES

55 To describe the presentation of OSSN and identify clinical features which distinguish it from
56 benign lesions and subsequently evaluate their recognisability.

57

58 DESIGN, SETTING AND PARTICIPANTS

59 Prospective multi-centre study in Kenya from July 2012 through July 2014 of 496 adults
60 presenting with conjunctival lesions.

61

62 EXPOSURES

63 Comprehensive history, slit lamp examination and photography before excision biopsy.

64 Frequency of clinical features in OSSN and benign lesions recorded. One histopathologist
65 examined all specimens. Six additional masked ophthalmologists independently examined
66 photographs from 100 participants and assessed clinical features.

67

68 MAIN OUTCOMES AND MEASURES

69 Proportions and means were compared using Chi-square, Fisher's exact test or t-test as
70 appropriate. Inter-observer agreement was estimated using Kappa statistic. Examiners'
71 assessments were compared to a reference.

72

73 RESULTS

74 Among 496 participants, OSSN was the most common (38%) histological diagnosis,
75 followed by pterygium (36%) and actinic keratosis (19%). OSSN cases were slightly older
76 and tended to have lower levels of education than benign ones. Females predominated

77 (67% of OSSN vs 64% of benign lesions; $P = .65$). HIV-infection was common among OSSN
78 cases (74%). The commonest location was the nasal limbus (61% OSSN vs 78% benign
79 lesions; $P < .001$). Signs more frequent in OSSN included; feeder vessels, odds ratio [OR],
80 5.8 [95%CI, 3.2-10.5]; moderate inflammation, OR, 3.5 [95%CI,1.8-6.8]; corneal
81 involvement, OR, 2.7 [95%CI,1.8-4.0]; leukoplakia, OR, 2.6 [95%CI,1.7-3.9]; papilliform
82 surface, OR, 2.1 [95%CI,1.3-3.5]; pigmentation, OR, 1.5 [95%CI, 1.0-2.2]; temporal location,
83 OR, 2.0 [95%cl, 1.2-3.2]; circumlimbal location, (7.0% vs 0.3%; $P < .001$); severe
84 inflammation (6.0% vs 0.3%; $P < .001$) and larger mean [SD] diameter (6.8 [3.2]mm vs
85 4.8[2.8]mm; $P < .001$). All OSSN signs were also observed in benign lesions. There was
86 slight to fair inter-observer agreement in assessment of most signs and diagnosis (Kappa,
87 0.1-0.4). The positive predictive value of clinical appearance in identifying OSSN was 54%
88 (interquartile range, 51-56) from photographs where prevalence was 32%.

89

90 CONCLUSIONS AND RELEVANCE

91 With overlapping phenotypes and modest inter-observer agreement, OSSN and benign
92 conjunctival lesions are not reliably distinguished clinically. Point-of-care diagnostic tools
93 may help.

94

95 **Background**

96 Ocular surface squamous neoplasia (OSSN) is a spectrum of pathology ranging from non-
97 invasive intra-epithelial dysplasia of the conjunctiva and cornea (CCIN) to invasive
98 squamous cell carcinoma.¹ Worldwide, the incidence rate of OSSN is highest in the southern
99 hemisphere (16⁰S) with the peak occurring in Africa.²

100

101 The gold standard for diagnosis of OSSN is histopathology; however, the availability of this
102 service is limited in Africa.^{3,4} The decision to excise conjunctival lesions usually depends on
103 the clinical impression. Most lesions are excised without subsequent histopathological
104 confirmation of the diagnosis or information on tumour involvement of the excision margins.
105 Even in countries with good access to pathology services, many lesions suspected of being
106 OSSN are treated without histological confirmation of the diagnosis. In 2003 a standard of
107 care survey in the USA showed that 51% of respondents always perform biopsies before
108 instituting therapy for suspected OSSN lesions.⁵ This proportion was unchanged when the
109 same survey was repeated in 2012.⁶ There are several reports from other regions where
110 primary treatment for suspected OSSN tumours is provided using topical agents (mitomycin
111 C, 5-fluorouracil, and interferon α 2b) without excision for histopathological diagnosis.⁷⁻¹⁰ The
112 rationale for this practice is to reduce the complications of excision such as limbal stem cell
113 deficiency with large lesions or symblepharon. Population surveys to determine the
114 prevalence of pinguecula or pterygium also rely on a clinical diagnosis to distinguish them
115 from OSSN and other benign lesions.¹¹⁻¹³

116

117 Several studies have tried to identify clinical features that may distinguish OSSN. A study in
118 Tanzania found that OSSN lesions had a shorter mean duration than benign lesions (3.7
119 months vs 8.8 months; $P = .03$) while feeder vessels were more frequently associated with
120 OSSN than benign lesions ($P = .03$).¹⁴ Male gender, temporal and superior locations, lack of
121 corneal involvement, papillomatous and nodular appearance were associated with higher-

122 grade OSSN lesions in a US study.¹⁵ OSSN lesions in HIV-infected individuals may be more
123 likely to be of a higher grade of malignancy than HIV-negative patients.¹⁶

124

125 The aim of this study was to describe the clinical presentation of OSSN in Kenya and
126 determine what clinical features might help to distinguish it from benign lesions. The main
127 focus was on the frequency of clinical features in OSSN that could help to differentiate
128 OSSN from other benign conjunctival lesions in this setting and the inter-observer variability
129 in the recognition of these features.

130

131

132 **Methods**

133 *Ethical Approval*

134 This study was part of an integrated set of investigations into OSSN in Kenya. It was formally
135 reviewed and approved by the Kenyatta National Hospital – University of Nairobi Ethics and
136 Research Committee and the London School of Hygiene and Tropical Medicine Ethics
137 Committee. This study adhered to the tenets of the Declaration of Helsinki. All participants
138 gave informed written consent to take part in the study before enrolment and did not receive
139 a stipend to participate.

140

141 *Participants*

142 Recruitment was between July 2012 and July 2014 in four eye care centers: Kenyatta
143 National Hospital in Nairobi, PCEA Kikuyu Eye Unit about 25 kilometres (km) from Nairobi in
144 Central Kenya, Kitale district hospital in the north Rift Valley 490km from Nairobi and Sabatia
145 Eye Hospital 300km from Nairobi in the western highlands bordering Lake Victoria. We
146 prospectively recruited all consenting, consecutive self-presenting adult patients (at least 18
147 years of age) with any conjunctival lesion (first presentation or a recurrence) suspected to be
148 OSSN scheduled for surgery. Pregnant women and breastfeeding mothers were excluded.

149

150 *Clinical Assessment*

151 A comprehensive history was taken using a structured questionnaire and the eyes were
152 examined with a slit lamp. The widest diameter of the lesion was measured using the slit
153 lamp beam and scale. A pair of photographs of each lesion was taken, one in primary gaze
154 and the other with the lesion in the center of the field. We used a Nikon D90 digital camera
155 with Micro Nikkor 105mm F2.8 AFS zoom lens and the R1 close up speedlight. All photos
156 were taken at 1:1 magnification ratio.

157

158 *Surgery and Histopathology*

159 All lesions were excised under local anaesthetic using an operating microscope with a 3mm
160 clear margin. Cryotherapy was not applied as the participants were further invited to enroll in
161 a treatment trial post-operatively. Specimens were placed directly into buffered formalin and
162 subsequently examined at the histopathology laboratory at the MP Shah Hospital, Nairobi.

163 One pathologist examined all the histology slides. Participants with mild, moderate or severe
164 conjunctival intraepithelial neoplasia (CIN I, II, III respectively) together with any who had
165 carcinoma-in-situ (CIS) and invasive squamous cell carcinoma were classified as having
166 OSSN. A three-grade system was used to classify carcinomas histologically as well,
167 moderately and poorly differentiated after the American Joint Committee on Cancer
168 (AJCC).¹⁷ Benign lesions included pterygium, actinic keratosis, papillomas, pyogenic
169 granulomas, nevi and rhinosporidiosis. The diagnosis of actinic keratosis was based on the
170 presence of elastotic stromal degeneration, acanthosis, hyperkeratosis and parakeratosis in
171 the presence of normal cellular polarity. By the accepted criteria for dysplasia, such lesions
172 were classified as CIN only if there is loss of polarity.

173

174

175 Cases of OSSN were invited to enroll in a case-control study that involved testing for HIV
176 and CD4 count. HIV was initially tested using Vironostika antigen/antibody kit then later
177 changed to rapid tests using Alere Determine HIV-1/2 Ag/Ab and Trinity Unigold. CD4 count

178 was tested using FacsCount (Becton Dickinson) USA. Those with benign lesions were not
179 tested. Voluntary testing and counselling was offered at the health facility.

180

181 *Inter-observer Study*

182 To determine the inter-observer variability in the assessment of the clinical features six final
183 year ophthalmology residents in the University Of Nairobi Department Of Ophthalmology at
184 Kenyatta National Hospital independently assessed photographs from the last 100
185 consecutive participants enrolled into the study from one center. They were masked to the
186 diagnosis. Images were projected onto a screen. The clinical case-mix was the same in this
187 sample of patients compared to the whole dataset that included patients from all the four
188 study centers. Cases with features that may suggest malignancy such as very large tumours
189 filling the orbit were excluded from this assessment. The graders were asked to determine if
190 each feature was either present, absent or difficult to determine.

191

192 *Statistical Analysis*

193 Data was managed in an Access database (Microsoft), cleaned and transferred into STATA
194 version 12.1 (StataCorp, College Station, Texas, USA) for analysis. In this analysis we
195 compared the clinical features of OSSN and benign lesions. Large orbital tumours and non-
196 OSSN malignancies were excluded. Categorical variables were compared using the
197 Pearson's chi-square test, odds ratios (ORs) or Fisher's exact test where appropriate.
198 Logistic regression was used to obtain adjusted ORs. To determine whether continuous
199 variables were normally distributed we generated Q-Q plots and compared the variances in
200 both groups using the standard deviation test. Where the deviations differed the t-test was
201 conducted with unequal variances.

202

203 The inter-observer agreement between graders was compared using the kappa (K) statistic
204 without weighting and graded using the Landis & Koch method as poor, slight, fair,
205 moderate, substantial or almost perfect.¹⁸ To calculate an average value, the kappa statistics

206 for each grader were transformed to Z scores using the Fisher Z transformation, averaged,
207 and then back-transformed to kappa.

208

209

210 **Results**

211 Five hundred and thirty-seven participants with conjunctival lesions were enrolled. Histology
212 reports were available for 496 participants. Eighteen tissue specimens were autolysed on
213 arrival at the pathology lab perhaps from poorly reconstituted formalin (one was a batch of
214 16 from one center) and 22 were presumed lost in transit. Seven (1.4%) were large orbital
215 tumours. A total of 488 participants were therefore included in the analysis of clinical
216 features.

217

218 *Histopathological Diagnosis*

219 OSSN was the most common type of ocular surface lesion (38%) (eTable 1 in the
220 supplement). This was followed by pterygium (36%) and actinic keratosis (19%), which were
221 the most common benign lesions. All stages of OSSN were seen with the most frequent
222 being moderately differentiated squamous cell carcinoma. There was one case of
223 sarcomatoid spindle cell carcinoma and a wide range of benign lesions.

224

225 *Demographic Characteristics*

226 The demographic characteristics of participants, subdivided by the pathology type are shown
227 in Table 1. About two-thirds were female (65%), with no difference between OSSN and
228 benign lesions. Most individuals presenting with conjunctival lesions were young to middle
229 aged adults (mean [SD] age, 39 [11.3] years). Participants with OSSN were slightly older
230 than those with benign lesions ($P = .002$), more likely to be widowed, and to have a lower
231 level of education. Those who did not have any formal education had the highest risk of
232 OSSN after adjusting for age and marital status.

233

234 *Clinical History*

235 The primary symptoms at presentation are shown in [eTable 2 in the supplement](#). Overall, the
236 presenting symptoms were similar by disease group ($P = .14$). The most frequent presenting
237 complaint was a lump or swelling (67%) followed pain (12%), redness (6%) and itchiness
238 (5%).

239

240 Additional information on the clinical history is presented in [eTable 3 in the supplement](#).

241 Median duration from first developing symptoms to presentation was longer for OSSN than
242 benign tumours (8 months vs 5 months; $P = .03$) and a history of prior conjunctival excision
243 was more frequent in OSSN than benign lesions (18% vs 6%; $P < .001$). The mean [SD]
244 number of prior excision surgeries where this had taken place was however similar in both
245 groups (1.4 [0.8] vs 1.3 [0.7]; $P = .66$). There was no evidence of a difference between
246 OSSN and benign lesions in terms of a family history of eye cancer or cancer at another site.

247

248 There was strong evidence that participants with OSSN had longer sun exposure in their
249 current ($P = .02$) and previous ($P = .003$) occupation, but little evidence that they had a
250 current predominantly outdoor occupation (64% vs 57%; $P = .14$), or worked outdoors in
251 previous employment (57% vs 48%; $P = .22$). There was no difference in the proportion who
252 wore hats or sunglasses, or who smoked cigarettes. However, among smokers, the mean
253 [SD] number of cigarettes smoked daily was higher among OSSN patients (12 [11] vs 7[6], P
254 = .03).

255

256 Of 133 OSSN patients tested for HIV, 98 (74%) were positive. Median CD4 count of 91
257 patients with OSSN was 265 cells/mm³ (interquartile range, 125-670 cells/mm³). Some
258 participants did not return for histology results after surgery and thus were not tested for HIV
259 or CD4. Participants with OSSN were more likely to be on ART than those with benign
260 lesions (38% vs 15%; $P < .001$). There was little evidence of a difference ($P = .30$) in mean
261 [SD] duration of ART use in those with OSSN (2.9 [3.0] years) compared to those with

262 benign lesions (3.5[2.9] years). According to the Kenya Ministry of Health HIV guidelines,
263 HIV-infected patients with $CD4 \leq 350$ cells/mm³ at first contact would be eligible for ART.¹⁹ It
264 is difficult to know how many of our patients were eligible for ART as they were already in
265 various stages of HIV care.

266

267 *Clinical Features*

268 Clinical features are described in [Table 2](#) and illustrated in [Figures 1&2](#). There were a wide
269 variety of presentation patterns for each type of OSSN. We illustrate this with a range of
270 moderately differentiated squamous cell carcinoma tumours in [Figure 1, F-O](#). Overall, OSSN
271 lesions were larger than benign lesions (mean [SD] diameter 6.8 [3.2] mm vs 4.8mm [2.8], P
272 $< .001$). All the features seen in OSSN also occurred in benign lesions ([Table 2](#)) and this
273 overlap is illustrated in [Figure 2](#). OSSN lesions were more likely to be at the temporal limbus
274 (28% vs 16%; $P = .002$), circumlimbal (7.0% vs 0.3%; $P < .001$), to have severe
275 inflammation ($P < .001$) and leukoplakia (72% vs 50%; $P < .001$). A gelatinous appearance
276 occurred with almost equal frequency in both groups, while a fibrovascular appearance was
277 more frequent in benign lesions and a papilliform appearance in OSSN. OSSN was more
278 likely to be pigmented, have a feeder vessel and involve the cornea. Regional
279 lymphadenopathy was rare ($n=7$, 1.5%) in OSSN even in those with large orbital tumours.

280

281 *Patients with large orbital tumours*

282 All seven participants with large orbital tumours had squamous cell carcinoma. Four were
283 female and 3 were male. Their age ranged from 30 to 85 years. Only one had prior excision
284 surgery, although no histology report was available. The tumours had been first noted 7
285 months to 15 years earlier. Five had HIV infection and 3 were on ART. Despite having large
286 tumours for a long time only 2 of them had regional lymphadenopathy.

287

288 *Inter-observer variation in recognition of clinical features*

289 Inter-observer variation is described in **eTable 4 in the supplement**. Overall there was fair to
290 moderate agreement in assessment of most signs and the clinical diagnosis. Most features
291 were easily recognized by the graders. The proportions of features they recognized were
292 fairly similar to an experienced examiner. Using clinical features to make a diagnosis of
293 OSSN had a median sensitivity of 86% (interquartile range, 81-88), specificity of 60%
294 (interquartile range, 53-69) and positive predictive value of 54% (interquartile range, 51-56)
295 among the six examiners (**eTable 5 in the supplement**).

296

297

298 **Discussion**

299 There appears to be a tendency to treat presumed OSSN without a tissue diagnosis.
300 However, we found a high degree of overlap in the clinical features of OSSN and benign
301 lesions. Although some features were more frequent in OSSN than the benign group, they
302 still occurred at a fairly high frequency in the benign group. In our view, the differences are
303 insufficient to depend upon clinical features as an indicator of the underlying diagnosis.
304 Moreover, there was only modest ($k=0.4$) inter-observer agreement in the assessment of the
305 diagnosis and a positive predictive value (54%) no better than chance when using clinical
306 features to make the diagnosis. The difficulty observed in determining surface appearance
307 may be partly attributed to the lack of a stereoscopic view from photographs. The agreement
308 in determining the presence of most clinical features was better than that for overall
309 diagnostic classification into OSSN or benign.

310

311 The age and sex distribution of OSSN patients was consistent with prior series from Africa,
312 where young adults and especially women predominate.^{2, 20} In temperate regions it is
313 predominantly a disease of older males.^{21, 22} There was no difference in the sex distribution
314 of OSSN and benign lesions. Higher education may increase awareness and earlier health
315 seeking behaviour. Median duration before presentation did not however conform to this
316 trend, and possibly showed the opposite of what has been previously reported.

317

318 The medical history of patients with OSSN and benign lesions is essentially similar. The
319 difference in occupational history with a longer exposure to solar ultraviolet radiation (UVR)
320 in those with OSSN than benign lesions is consistent with UVR being a major risk factor for
321 OSSN.² There was also a heavier exposure to cigarette smoking with OSSN lesions, which
322 has so far not been clearly described as a risk factor for OSSN.

323

324 Although some clinical features showed differences between OSSN and benign lesions, it
325 may be difficult to tell the two apart. For instance, OSSN lesions were larger than benign
326 lesions but a 2.0mm difference between 6.8mm and 4.8mm is relatively small. A
327 circumlimbal pattern was more frequent in OSSN; however, it only occurred in 3% of the
328 conjunctival lesions. While OSSN was twice as likely to be temporal, 16% of benign lesions
329 were located temporally, compared to 28% of OSSN lesions. Such a difference in proportion
330 is difficult to rely on in the clinical setting.

331

332 The preponderance of nasal conjunctival lesions is consistent with earlier reports, and may
333 be due to the previously described observation that incident temporal sunlight is focused
334 nasally with a 20-fold magnification in intensity.²³ Pterygia and actinic keratosis are
335 considered pre-malignant and have some similarities with OSSN in their pathophysiology
336 including association with solar UV radiation, p53 gene mutation and human papilloma virus
337 (HPV).²⁴⁻²⁷ Being on the same causal pathway may also explain the overlap of clinical
338 features. Further, we would also expect benign changes to occur before malignant ones.
339 This may explain why OSSN cases were older than the benign cases most of whom had
340 pterygia or actinic keratosis.

341

342 Differences between OSSN and benign lesions in the proportions of moderate and severe
343 inflammation ($P < .001$) may not in isolation be easily applied in the clinical setting. OSSN
344 was more likely to show leukoplakia than benign lesions, however 50% of benign lesions

345 also had it. This situation is also seen with other features like the lesion surface
346 appearance, pigmentation, feeder vessels and corneal involvement in **Table 2 & Figure 2**.

347

348 This study has a number of limitations. The six examiners in the inter-observer component
349 did not have a full history, which may help to inform the clinical diagnosis, nor did they
350 assess the lesions at the slit lamp as this would have been logistically impossible. Secondly,
351 this was a hospital-based study, which may introduce selection bias in the types of patients
352 seen. However the objective of the study was to compare OSSN and benign lesions
353 presenting to clinicians in a healthcare facility setting, so this potential bias would not affect
354 comparability of the two types of disease. Finally, distinguishing pterygia and OSSN by
355 histopathology is sometimes difficult. Studies in Australia and USA found histopathological
356 features of OSSN in 9.8% and 1.7% respectively of lesions previously classified as
357 pterygia.^{28, 29}

358

359 In conclusion the clinical features of OSSN and benign conjunctival lesions overlap. Both
360 disease groups have common pathophysiological mechanisms and this may explain their
361 overlapping clinical appearance. Although individual features are identified by different
362 examiners with reasonable consistency, they do not reliably distinguish the two disease
363 groups. Examination of photographs alone cannot replace clinical examination and biopsy,
364 indicating that teleophthalmology approaches for the diagnosis of OSSN require more study.
365 Therefore in the African context where the range of risk factors is perhaps wider and the
366 clinical behaviour of the disease more aggressive compared to temperate regions we
367 conclude that biopsy should be performed before treatment. The occurrence of malignant
368 changes described in pterygia and other benign lesions further underscores the need for
369 histopathology.

370

371

372 **Acknowledgments:** The authors have no conflict of interest disclosures. SG received
373 funding from the British Council for Prevention of Blindness (BCPB) fellowship programme.
374 MJB is supported by The Wellcome Trust (Grant Number 098481/Z/12/Z). The funding
375 organizations had no role in the design or conduct of this research. SG and MJB had full
376 access to all the data in the study and take responsibility for the integrity of the data and the
377 accuracy of the data analysis.

378 **Author contributions:**

379 *Study concept and design:* Gichuhi, Sagoo, Weiss, Burton
380 *Acquisition, analysis, or interpretation of data:* All authors
381 *Drafting of the manuscript:* Gichuhi, Sagoo, Weiss, Burton
382 *Critical revision of the manuscript for important intellectual content:* All authors
383 *Statistical analysis:* Gichuhi, Weiss, Burton
384 *Obtained funding:* Gichuhi, Weiss, Burton
385 *Administrative, technical, or material support:* Macharia, Kabiru, Zindamoyen, Rono,
386 Ollando, Wanyonyi, Wachira, Munene, Sagoo, Weiss, Burton *Study supervision:* Weiss,
387 Burton

388

389 **Conflict of interest disclosures:**

390 All authors have completed and submitted the ICMJE Form for Disclosure of Potential
391 Conflicts of Interest and none were reported.

392

393

394 **References**

395
396 1. Lee GA, Hirst LW. Ocular surface squamous neoplasia. *Surv Ophthalmol.* 1995;39(6):429-450.
397 2. Gichuhi S, Sagoo MS, Weiss HA, Burton MJ. Epidemiology of ocular surface squamous
398 neoplasia in Africa. *Trop Med Int Health.* 2013;18(12):1424-1443.
399 3. Adesina A, Chumba D, Nelson AM, et al. Improvement of pathology in sub-Saharan Africa.
400 *Lancet Oncol.* 2013;14(4):e152-157.
401 4. Rambau PF. Pathology practice in a resource-poor setting: Mwanza, Tanzania. *Arch Pathol*
402 *Lab Med.* 2011;135(2):191-193.
403 5. Stone DU, Butt AL, Chodosh J. Ocular surface squamous neoplasia: a standard of care survey.
404 *Cornea.* 2005;24(3):297-300.
405 6. Adler E, Turner JR, Stone DU. Ocular surface squamous neoplasia: a survey of changes in the
406 standard of care from 2003 to 2012. *Cornea.* 2013;32(12):1558-1561.
407 7. Nutt RJ, Clements JL, Dean WH. Ocular surface squamous neoplasia in HIV-positive and HIV-
408 negative patients and response to 5-fluorouracil in Angola. *Clin Ophthalmol.* 2014;8:2435-
409 2440.
410 8. Russell HC, Chadha V, Lockington D, Kemp EG. Topical mitomycin C chemotherapy in the
411 management of ocular surface neoplasia: a 10-year review of treatment outcomes and
412 complications. *Br J Ophthalmol.* 2010;94(10):1316-1321.
413 9. Besley J, Pappalardo J, Lee GA, Hirst LW, Vincent SJ. Risk factors for ocular surface squamous
414 neoplasia recurrence after treatment with topical mitomycin C and interferon alpha-2b. *Am J*
415 *Ophthalmol.* 2014;157(2):287-293 e282.
416 10. Nanji AA, Moon CS, Galor A, Sein J, Oellers P, Karp CL. Surgical versus medical treatment of
417 ocular surface squamous neoplasia: a comparison of recurrences and complications.
418 *Ophthalmology.* 2014;121(5):994-1000.
419 11. Le Q, Xiang J, Cui X, Zhou X, Xu J. Prevalence and associated factors of pinguecula in a rural
420 population in Shanghai, Eastern China. *Ophthalmic Epidemiol.* 2015;22(2):130-138.
421 12. Marmamula S, Khanna RC, Rao GN. Population-based assessment of prevalence and risk
422 factors for pterygium in the South Indian state of Andhra Pradesh: the Andhra Pradesh Eye
423 Disease Study. *Invest Ophthalmol Vis Sci.* 2013;54(8):5359-5366.
424 13. Viso E, Gude F, Rodriguez-Ares MT. Prevalence of pinguecula and pterygium in a general
425 population in Spain. *Eye (Lond).* 2011;25(3):350-357.
426 14. Nguena MB, van den Tweel JG, Makupa W, et al. Diagnosing ocular surface squamous
427 neoplasia in East Africa: case-control study of clinical and in vivo confocal microscopy
428 assessment. *Ophthalmology.* 2014;121(2):484-491.
429 15. Kao AA, Galor A, Karp CL, Abdelaziz A, Feuer WJ, Dubovy SR. Clinicopathologic correlation of
430 ocular surface squamous neoplasms at bascom palmer eye institute: 2001 to 2010.
431 *Ophthalmology.* 2012;119(9):1773-1776.
432 16. Makupa, II, Swai B, Makupa WU, White VA, Lewallen S. Clinical factors associated with
433 malignancy and HIV status in patients with ocular surface squamous neoplasia at Kilimanjaro
434 Christian Medical Centre, Tanzania. *Br J Ophthalmol.* 2012;96(4):482-484.
435 17. American Joint Committee on Cancer. Carcinoma of the conjunctiva. In: Edge SB, Byrd DR,
436 Compton CC, Fritz AG, Greene FL, Trotti A, eds. *Cancer staging handbook.* 7 ed. New York:
437 Springer; 2010:600-602.
438 18. Landis JR, Koch GG. The measurement of observer agreement for categorical data.
439 *Biometrics.* 1977;33(1):159-174.
440 19. National AIDS/STI Control Program (NAS COP). Guidelines on Use of Antiretroviral Drugs for
441 Treating and Preventing HIV Infection: A rapid advice; 2014.
442 20. Tiong T, Borooah S, Msosa J, et al. Clinicopathological review of ocular surface squamous
443 neoplasia in Malawi. *Br J Ophthalmol.* 2013;97(8):961-964.

- 444 **21.** Kim BH, Kim MK, Wee WR, Oh JY. Clinical and pathological characteristics of ocular surface
445 squamous neoplasia in an Asian population. *Graefes Arch Clin Exp Ophthalmol.*
446 2013;251(11):2569-2573.
- 447 **22.** Tunc M, Char DH, Crawford B, Miller T. Intraepithelial and invasive squamous cell carcinoma
448 of the conjunctiva: analysis of 60 cases. *Br J Ophthalmol.* 1999;83(1):98-103.
- 449 **23.** Coroneo M. Ultraviolet radiation and the anterior eye. *Eye Contact Lens.* 2011;37(4):214-
450 224.
- 451 **24.** Liu T, Liu Y, Xie L, He X, Bai J. Progress in the pathogenesis of pterygium. *Curr Eye Res.*
452 2013;38(12):1191-1197.
- 453 **25.** Chalkia AK, Spandidos DA, Detorakis ET. Viral involvement in the pathogenesis and clinical
454 features of ophthalmic pterygium (Review). *Int J Mol Med.* 2013;32(3):539-543.
- 455 **26.** Di Girolamo N. Association of human papilloma virus with pterygia and ocular-surface
456 squamous neoplasia. *Eye (Lond).* 2012;26(2):202-211.
- 457 **27.** Dushku N, Hatcher SL, Albert DM, Reid TW. p53 expression and relation to human
458 papillomavirus infection in pingueculae, pterygia, and limbal tumors. *Arch Ophthalmol.*
459 1999;117(12):1593-1599.
- 460 **28.** Hirst LW, Axelsen RA, Schwab I. Pterygium and associated ocular surface squamous
461 neoplasia. *Arch Ophthalmol.* 2009;127(1):31-32.
- 462 **29.** Oellers P, Karp CL, Sheth A, et al. Prevalence, treatment, and outcomes of coexistent ocular
463 surface squamous neoplasia and pterygium. *Ophthalmology.* 2013;120(3):445-450.

464

465

466 **Figure titles and legends**

467 **Figure 1. Five grades of inflammation associated with OSSN are shown in A-E. Various clinical**
468 **features seen in moderately differentiated squamous cell carcinoma are shown from F to O. F-**
469 **K shows different tumour surface appearances and various growth patterns are seen in L-O.**
470 **(A)** No inflammation; **(B)** Minimal inflammation with leukoplakia and brown pigmentation;n **(C)** Mild
471 inflammation with leukoplakia; **(D)** Moderate inflammation with leukoplakia; **(E)** Severe inflammation
472 with leukoplakia; **(F)** Leukoplakia – patches of keratosis visible as white adherent plaques. Feeder
473 vessels (distinctly dilated blood vessels larger than the rest of conjunctival vessels) are also shown by
474 yellow arrows; **(G)** Erythoplakia – a red subconjunctival popular haemorrhage-like appearance; **(H)**
475 Gelatinous appearance; **(I)** Fibrovascular appearance; **(J)** Papilliform appearance with markedly large
476 feeder vessels (yellow arrows); **(K)** Brown pigmentation; **(L)** circumlimbal lesion; **(M)** pedunculated
477 lesion;**(N)** Extensive corneal involvement with orbital disease; **(O)** Symblepharon.

478

479

480 **Figure 2. Overlapping clinical features of OSSN and benign/pre-malignant lesions.**

481 A gelatinous surface with pigmentation in **(A)** a moderately differentiated squamous cell carcinoma
482 and **(B)** a pterygium. A papilliform surface in **(C)** CIN2 and **(D)** a squamous papilloma. Note that the
483 squamous papilloma in addition shows multiple feeder vessels. A fibrovascular appearance in **(E)** a
484 moderately differentiated squamous cell carcinoma and **(F)** a pterygium. The pterygium has some
485 brown pigmentation also seen on the temporal side of the same eye. Leukoplakia with moderate
486 inflammation in **(G)** a well differentiated squamous cell carcinoma and **(H)** an actinic keratosis
487 showing. Brown pigmentation in **(I)** CIN3 and **(J)** a nevus.

488 **Table 1. Demographic characteristics of participants with OSSN and benign conjunctival lesions. This includes orbital disease.**
 489

Demographic feature	OSSN N=187	Benign N=308	OSSN vs Benign lesions			
			Crude OR (95% CI)	P value	Adj OR ^a (95% CI)	P value
Sex, No. (%)						
Male	62 (33.0)	110 (36.0)	1 [Reference]		1 [Reference]	
Female	125 (67.0)	198 (64.0)	1.1 (0.8 - 1.6)	.56	1.1 (0.8 - 1.6)	.65
Age in years, mean (SD), y	41 (11.6)	38 (10.9)	NA	.002 ^b	NA	NA
Marital status, No. (%)						
Single	30 (16.0)	42 (14.0)	1 [Reference]		1 [Reference]	
Married	123 (66.0)	231 (75.0)	0.8 (0.4 - 1.3)	.04	0.5(0.3 - 0.9)	.05
Divorced or Separated	11 (6.0)	18 (6.0)	0.9 (0.4 - 2.1)		0.5 (0.2 - 1.3)	
Widowed	23 (12.0)	17 (6.0)	1.9 (0.9 - 4.2)		0.9 (0.4 - 2.2)	
Highest education level, No. (%)						
More than secondary	17 (9.0)	66 (21.0)	1 [Reference]		1 [Reference]	
Completed secondary school	58 (31.0)	85 (28.0)	2.7(1.4 - 5.0)		2.7 (1.4 - 5.1)	
Some secondary school	13 (7.0)	37 (12.0)	1.4 (0.6 - 3.1)	.001	1.4 (0.6 - 3.4)	<.001
Completed primary school	57 (30.0)	74 (24.0)	3.0 (1.6 - 5.8)		3.1 (1.6 - 5.9)	
Some primary school	24 (13.0)	38 (12.0)	2.5 (1.2 - 5.2)		2.4 (1.1 - 5.3)	
None	18 (10.0)	8 (3.0)	8.7 (2.9 - 26.5)		10.8 (3.3 - 34.8)	

490 Abbreviations: OSSN, ocular surface squamous neoplasia; SD, standard deviation; NA, not applicable
 491 ^a adjusted for education, age group and marital status. Sex did not change the multivariable model so it was not included.
 492 ^b t test
 493
 494

495 **Table 2. Comparison of the clinical features of OSSN with benign conjunctival lesions on slit**
 496 **lamp examination.**
 497

Clinical feature	OSSN	Benign lesions	OSSN vs benign	
	N=180 No. (%)	N=308 No. (%)	OR (95% CI)	P value
Lesion location				
nasal limbus	110 (61.0)	241 (78.0)	0.4 (0.3 - 0.7)	<.001
temporal limbus	50 (28.0)	50 (16.0)	2.0 (1.2 - 3.2)	.002
superior limbus	2 (1.0)	2 (0.7)	1.7 (0.1 - 23.9)	.59
inferior limbus	1 (0.6)	4 (1.3)	0.4 (0.0 - 4.3)	.43
circumlimbal	12 (7.0)	1 (0.3)	21.9 (3.2 - 940.2)	<.001
mostly corneal	1 (0.6)	0 (0.0)	∞	.19
both nasal & temporal limbus	3 (2.0)	1 (0.3)	5.2 (0.4 - 274.0)	.11
caruncle	0 (0.0)	3 (1.0)	0 (0.0 - 2.2)	.18
lid	1 (0.6)	6 (2.0)	0.3 (0.0 - 2.4)	.21
Inflammation at the lesion site				
none	21 (13.0)	74 (24.0)	1[Reference]	
minimal	50 (28.0)	111 (36.0)	1.6 (0.9 - 2.9)	
mild	46 (25.0)	71 (23.0)	2.3 (1.2 - 4.3)	<.001
moderate	51 (28.0)	51 (17.0)	3.5 (1.8 - 6.8)	
severe	12 (6.0)	1 (0.3)	42.3 (3.7 - 478.3)	
Leukoplakia	129 (72.0)	152 (50.0)	2.6 (1.7 - 3.9)	<.001
Erythroplakia	30 (17.0)	53 (17.0)	1.0 (0.6 - 1.6)	.88
Gelatinous appearance	121 (67.0)	188 (61.0)	1.3 (0.9 - 2.0)	.19
Fibrovascular appearance	18 (10.0)	81 (26.0)	0.3 (0.2 - 0.6)	<.001
Papilliform appearance	41 (23.0)	38 (12.0)	2.1 (1.3 - 3.5)	.003
Brown lesion pigmentation	96 (53.0)	133 (44.0)	1.5 (1.0 - 2.2)	.04
Lesion feeder vessels	163 (91.0)	195 (64.0)	5.8 (3.2 - 10.5)	<.001
Corneal involvement	115 (65.0)	121 (40.0)	2.7 (1.8 - 4.0)	<.001
Lesion diameter, mean (SD), mm	6.8 (3.2)	4.8 (2.8)	NA	<.001 ^b

498 Abbreviations: OSSN, ocular surface squamous neoplasia; SD, standard deviation; NA, not applicable; ∞ stands for infinite

499 ^a The numbers assessed may vary in different cells if the item assessed did not apply to all participants

500 ^b t-test

501



