Prognostic Utility of Whole-Genome Sequencing and Polymerase Chain Reaction Tests of Ocular Fluids in Postprocedural Endophthalmitis

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• PURPOSE: To associate detection of potential pathogen DNA in endophthalmitis with clinical outcomes.
• DESIGN: Prospective cohort study.
• METHODS: Patients in whom endophthalmitis was diagnosed following an intraocular procedure were recruited. Clinical outcome data from baseline, week-1, month-1, and month-3 visits were collected. Intraocular biopsy samples were cultured by standard methods. Quantitative polymerase chain reaction (qPCR) was performed for specific pathogens and whole-genome sequencing (WGS).
• RESULTS: A total of 50 patients (mean age 72 years old; 52% male) were enrolled. Twenty-four cases were culture-positive and 26 were culture-negative. WGS identified the cultured organism in 76% of culture-positive cases and identified potential pathogens in 33% of culture-negative cases. Month-1 and -3 visual acuities did not vary by pathogen-positive versus pathogen-negative cases as detected by either culture or WGS. Visual outcomes of *Staphylococcus epidermidis* endophthalmitis were no different than those of pathogen-negative cases, whereas the patients infected with other pathogens showed worse outcome. Higher baseline bacterial DNA loads of bacteria other than those of *S epidermidis* detected by WGS were associated with worse month-1 and -3 visual acuity, whereas the *S epidermidis* loads did not appear to influence outcomes. Torque teno virus (TTV) and Merkel cell polyomavirus (MCV) were detected by qPCR in 49% and 19% of cases, respectively. Presence of TTV at presentation was associated with a higher rate of secondary pars plana vitrectomy ($P = .009$) and retinal detachment ($P = .022$).
• CONCLUSIONS: The presence and higher load of bacteria other than *S epidermidis* detected by WGS or DNA from TTV by qPCR in ocular fluids is associated with worse outcomes in post-procedure endophthalmitis. (Am J Ophthalmol 2020;217:325–334. © 2020 Elsevier Inc. All rights reserved.)

ENDOPHTHALMITIS IS A SERIOUS SIGHT-threatening condition. Postsurgical endophthalmitis occurs following approximately 0.1% to 0.2% of cataract surgeries, with substantial loss of vision in many cases.[1] Although the incidence rate of endophthalmitis following intravitreal injections is low, ranging in various studies from 0.01% to 0.1%,[1,2] more than 6 million intravitreal injections were performed in 2013 in the United States alone.[3] Thus, the total burden of endophthalmitis is increasing substantially with thousands of cases occurring annually.

Currently, microbial culture remains the gold standard for detecting organisms associated with endophthalmitis. However, the Endophthalmitis Vitrectomy Study (EVS) reported only 291 of 420 cases (69.3%) as culture-positive, leaving more than 30% of the cases without a diagnosis.[4] Culture-positive rates may be even lower in post-injection endophthalmitis.[5] In a review of 27,735 consecutive intravitreal injections among 23 cases of endophthalmitis, 16 cultures (70%) were culture-negative. As the prognosis of endophthalmitis appears at least partially dependent on the causative organism,[6] the high rate of culture-negative cases suggests a need for a more sensitive modality for pathogen detection.[5]

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The past several years have seen rapid advances in whole-genome DNA sequencing technologies, including biome representational in silico karyotyping (BRiSK),[7] 454 pyrosequencing,[8] and Illumina polony sequencing (Illumina dye; Solexa, Cambridge, United Kingdom).[9] These technologies facilitate direct, massively parallel sequencing of single DNA molecules and can identify and characterize pathogens with unprecedented detail. One of the advantages of these technologies is the potential to identify potential pathogens without a priori knowledge of differential diagnoses. Deep DNA or whole-genome sequencing (WGS) was applied previously in a study of 14 patients with endophthalmitis along with 7 subjects receiving pars plana vitrectomy for noninflammatory disease that served as control[10] and was shown to have sufficient sensitivity for detection of cultured organisms. However, the utility of this approach in providing prognostic information for management of endophthalmitis has not been assessed to date.

Previously, using the BRiSK technique, the present authors demonstrated the presence of torque teno virus (TTV) in both culture-positive and particularly culture-negative endophthalmitis[11]; however, in that study, outcomes were not analyzed. The present study sought to apply WGS techniques as well as directed detection of small DNA viruses (ie, TTV and Merkel cell polyomavirus [MCV]) to a larger, prospective cohort of patients with endophthalmitis and determine whether these techniques provide additional prognostic information for the management of post-procedure endophthalmitis.

METHODS

• **PATIENT RECRUITMENT:** This was a prospective cohort study conducted by MidAtlantic Retina, The Retina Service of Wills Eye Hospital, Philadelphia, Pennsylvania, and University of Washington, Seattle, Washington. Consecutive patients with a clinical diagnosis of endophthalmitis following any intraocular procedure or surgery within 6 weeks of presentation (≥42 days) were invited to participate in the study, and informed written consent was obtained. This study was approved by the Institutional Review Board from both institutions and conducted in accordance with the Declaration of Helsinki.

All patients underwent either intraocular fluid biopsy (aqueous humor or vitreous "tap") or pars plana vitrectomy (PPV) on the day of recruitment according to standard-of-care protocol for their endophthalmitis. Vitreous tap was attempted, and an aqueous tap performed if vitreous tap was unsuccessful. The decision to perform a vitreous or aqueous tap or vitrectomy was made at the discretion of the physician.[4] Extensive clinical data including records of additional procedures or surgery and microbiology results were obtained on the day of recruitment (day 1), week 1, month 1, and month 3. The intraocular biopsy samples were sent to the microbiology laboratory for routine culture in participating centers. The remaining ocular samples were frozen at −80°C and sent frozen on dry ice to the University of Washington for further molecular testing.

Details of the laboratory methods used (DNA extraction, polymerase chain reaction [PCR], and WGS) are found in the Supplemental Material.

• **BIOINFORMATIC ANALYSES:** All analyses were performed using the Scalable Metagenomic Analysis Research Tool (SMART) bioinformatic pipeline.[12] Briefly, all genomic DNA sequences from the National Center for Biotechnology Information (NCBI) GenBank (housing more than 1×10^{11} base pairs of 3.3×10^{6} sequences from 9.25×10^{5} species) were retrieved and indexed by using 4-base pair hash table shards. A 1-pair permutation method was used to account for single nucleotide polymorphisms and sequencing errors. Each sequence was matched against all known sequences of bacteria, DNA viruses, and fungi by using a multiplex, parallel searching strategy and classifying them as mammalian, nonmammalian, unknown, and ambiguous readings. The bacterial load was defined as the median number of the presumed pathogen per recovered human genome by WGS, calculated as: [(total number of reads of the pathogen)/(total number of human reads) × (size of the human genome)/(size of the pathogen genome)]. The genome coverage of the lead pathogen was assessed by aligning the reads to the pathogen's reference genome from the NCBI reference sequence database (Refseq). Average alignment breadth was defined as the number of bases having at least 1 sample base aligned to it, divided by the size of the reference genome in bases. Average alignment depth was the depth divided by the length of regions with a depth of 1 or greater. Average genomic coverage was the total number of input bases divided by the length of the reference genome = [(the average alignment depth) × (the average alignment breadth)].

• **STATISTICAL ANALYSES:** The primary endpoint of the study was the VAs at months 1 and 3. Factors related to WGS, PCR results, and clinical outcomes (eg, age, sex, type of surgery, culture status, pathogen status [*Staphylococcus epidermidis* or other organisms], and TTV or MCV presence) were assessed using Fisher's exact test and Wilcoxon-rank sum depending on the variable type. Spearman correlation was used to evaluate the associations between bacterial load and VA. Given that VAs were not normally distributed, logistic regression was used to evaluate the associations between poor visual outcome and other covariates. Poor visual outcome was defined as 20/200 or worse. Any covariate with a P value less than .2 was force-entered into the multivariate logistic regression. Time to secondary PPV was evaluated using Kaplan-Meier with Fleming-Harrington weighted log rank test, which

TABLE 1. Baseline Demographic and Clinical Factors in Culture Positive and Negative Groups

	All (n = 50)	Cx Positive (n = 24)	Cx Negative (n = 26)	*P*-Value
Mean age (range)	72.3 (36-98)	66.5 (44-85)	72.4 (36-98)	.835
Male, n (%)	26 (52)	14 (58)	12 (46)	.413
Type of surgery (%)				
Cataract	8 (15)	2 (8)	6 (23)	.431
Intravitreal injection	30 (58)	16 (67)	14 (54)	.431
Glaucoma	3 (6)	1 (4)	0	.431
Retina	10 (19)	5 (21)	5 (19)	.431
Other	1 (2)	0	1 (4)	.431
Median time to presentation, days (range)	6 (1-42)	5 (1-30)	8 (1-42)	.139
Median baseline VA, logMAR (Snellen)	2.4 (HM)	2.4 (HM)	2.4 (HM)	.747
TTV presence (%)[a]	23/47 (49)	8/22 (36)	15/25 (60)	.146
MCV presence (%)[a]	9/47 (29)	4/22 (18)	5/25 (20)	1.000

Cx = culture; VA = visual acuity; HM = hand motion; TTV = torque teno virus; MCV = merkel cell polyomavirus.
Statistical analyses: Wilcoxon rank sum, Fisher's exact test.
[a]Presence of TTV or MCV tested in 47 samples due to limited sample quantities.

emphasizes the late or long-term differences between groups compared to the conventional log rank test.[13] In lieu of a Bonferroni correction, raw data are shown.[14] Analyses were performed using R software (R project, Vienna, Austria) or SPSS version 25.0 software (IBM, Armonk, New York).

RESULTS

A TOTAL OF 50 PATIENTS WERE ENROLLED. MEAN AGE WAS 72 (range, 36-98 years old) and 26 (52%) were male. Median time to presentation was 6 days (range, 1-42 days) following the intraocular procedure. The most common antecedent procedure was intravitreal injection (58%), followed by vitrectomy (19%), and cataract surgery (15%). Twenty-three samples were aqueous humor and 27 were vitreous samples (2 primary vitrectomy and 25 vitreous tap specimens). No significant differences were found in total WGS reads between aqueous and vitreous samples ($P = .970$) (Supplemental Table 1). A total of 48 patients were seen at follow-up week 1 visit, 45 at month 1 and 43 at month 3.

• **MICROBIAL CULTURE:** A total of 24 samples were found to be culture-positive and 26 were culture-negative. No significant differences in demographic or baseline clinical factors, including the specimen type, were found between culture-positive and culture-negative groups (Table 1). The organism most commonly cultured was coagulase-negative *Staphylococcus* spp (eg, *S epidermidis*), followed by other *Staphylococcus* and *Streptococcus* spp (Supplemental Table 2).

• **WHOLE-GENOME SEQUENCING:** A total of 46 samples underwent WGS (4 had insufficient DNA following culture), and 42 samples with appropriate WGS quality (ie, >1 million total reads) were included in the analyses (Supplemental Table 1). The median number of reads in 42 samples were 7,394,807 (mean, 8,994,157) ranging from 1,524,618 to 27,080,354. The median read count between culture-positive and culture-negative cases did not differ significantly ($P = .990$).

• **BACTERIAL LOAD:** The median bacterial load in culture-positive cases was 3.32 (mean, 53.50; range, 0.028-480) (Supplemental Table 1). The median bacterial load in WGS-positive but culture-negative cases (ie, presumed falsely cultured negative cases) was 1.44 (mean, 2.04; range, 0.35-6.19). This difference was not significant ($P = .644$). Among all samples, no significant differences between bacterial loads was found between aqueous and vitreous samples or between TTV and MCV positive versus negative groups.

• **AGREEMENT BETWEEN WGS AND CULTURE:** The predominant organism detected by WGS agreed with culture results in 32 cases (76%) (Table 2). WGS detected the cultured pathogen as the predominant organism (the highest number of reads of the nonmammalian reads) in 17 of 20 (85%) culture-positive samples. One sample cultured *S aureus* whereas WGS identified *Pseudomonas fluorescens* as the primary pathogen. Two samples that grew *S aureus* in culture did not yield any organism on WGS. One of these 2 samples was further tested with pan-bacterial and pan-fungal PCR at the University of Washington microbiology lab. The 16S PCR did not reveal any bacteria, and 28S PCR was positive for *Sporidiobolus johnsonii*. In 8 of 22 culture-negative cases (36%), WGS detected potential

TABLE 2. Agreement Between Culture and Whole Genome Sequencing (n = 42[a])

	Culture Positive	Culture Negative	Total
WGS positive	18 (69%, 90%)[d]	8[b] (31%, 36%)	26
WGS negative	2[c] (13%, 10%)	14 (88%, 64%)	16
	20	22	42

[a]Out of 50, 4 excluded due to limited sample size and 4 due to low quality WGS (<1 million reads).
[b]7 *S. epidermidis*, 1 *Pseudomonas fluorescens.*
[c]Both *S. aureus* positive by culture, none identified by WGS.
[d]One case of *S. aureus* by culture but *Pseudomonas fluorescens* by WGS; (row %, column %).

pathogens including *S epidermidis* (n = 7) and *P fluorescens* (n = 1). The remaining 14 culture-negative cases did not yield an organism on WGS.

The authors aligned *S epidermidis* readings found in 7 culture-negative cases from which WGS detected *S epidermidis* as the pathogen, in which a mean of 1,497,448 base pairs (60%) were covered in the genome, ranging from 269,234 to 5,454,168 base pairs (Figure 1). The high coverage rate strongly suggests that the *S epidermidis* organism was present in these samples and was not detected by culture (Supplemental Table 3).

• **VIRAL IDENTIFICATION BY PCR:** A total of 47 samples were tested for the presence of TTV and MCV by quantitative (q)PCR. TTV was detected in 23 of 47 cases (49%), 13 of 25 aqueous samples (52%) and 10 of 22 vitreous samples (45%) ($P = .773$ for detection differences between fluids). TTV was detected in 8 of 22 (36%) of bacterial culture-positive and in 15 of 25 (60%) of culture-negative cases ($P = .147$). Age, sex, procedure type, specimen type (aqueous vs vitreous), and culture status were not associated with the presence of TTV ($P > .05$). However, when culture status was divided into 3 groups (*S epidermidis* vs other bacteria-positive vs culture-negative), the cases that were infected with *S epidermidis* were 60% less likely to have TTV (odds ratio [OR], 0.60; 95% confidence interval [CI], 0.41-0.88; $P = .010$), and the cases infected with other organisms were 51% less likely to have TTV than culture-negative cases (OR, 0.51; 95% CI, 0.33-0.78; $P = .003$).

MCV was detected in 9 of 47 cases (19%). MCV was detected in 4 of 22 (18%) of culture-positive cases and in 5 of 25 (20%) of culture-negative cases ($P = 1$) (Supplemental Table 4). Age, sex, procedure type, specimen type (aqueous vs vitreous), and culture status were not associated with the presence of MCV ($P > .05$). Unlike TTV, the presence of MCV was not found to vary by causative bacteria.

• **CLINICAL OUTCOMES:** *Presence or absence of pathogen by culture or WGS does not predict outcome.* Baseline, month-1, and month-3 VAs per different molecular categories are listed in Supplemental Table 5. It is notable that a large range exists in overall VA within each category (Supplemental Figures 1-4). Month-1 VA trended toward better VA in culture-negative cases compared to those in culture-positive cases (median VA, 0.4 vs 1.3), but this was not statistically significant ($P = 0.124$) (Supplemental Figure 1). Similarly, no statistically significant differences were found between the month-1 visual outcome of pathogen-positive versus negative cases detected as defined by WGS results (0.5 vs 0.5; $P = .448$). No significant differences in month-3 VAs were found between culture-positive and culture-negative cases (median 0.5 vs 0.4; $P = .237$), nor by cases defined by WGS results (median 0.4 vs 0.5; $P = .987$).

Culture- and WGS-negative cases and S epidermidis*-positive cases have superior clinical outcomes compared cases due to other pathogens.* Cases were divided into *S epidermidis*-positive cases by culture (n = 10), other culture-positive cases (ie, non-*S epidermidis*) (n = 14), and culture-negative cases (n = 26). *S epidermidis* cases behavior was similar to that of culture-negative cases, whereas other culture-positive samples had worse VA outcomes (Supplemental Figure 2). There were no statistically significant differences in month-1 VA outcomes between those of *S epidermidis* and the culture-negative cases (median 0.5 vs 0.4; $P = .947$). However, the combined VAs of culture-negative and *S epidermidis* cases were significantly better than other positive cases at month 1 (median 0.4 vs 2.0; $P = 0.021$). There were no statistically significant differences in the month-3 VA outcome between *S epidermidis* and those of culture-negative cases (median 0.7 vs 0.4; $P = 0.469$). Unlike month-1 results, the combined VA of culture-negative and *S epidermidis* cases were not significantly different from other positive cases (median 0.5 vs 0.5; $P = 0.295$). Analyzing by the pathogen status detected by WGS instead of conventional culture revealed similar results (Appendix).

The presence of small viruses (TTV or MCV) was not associated with significant differences in visual outcome

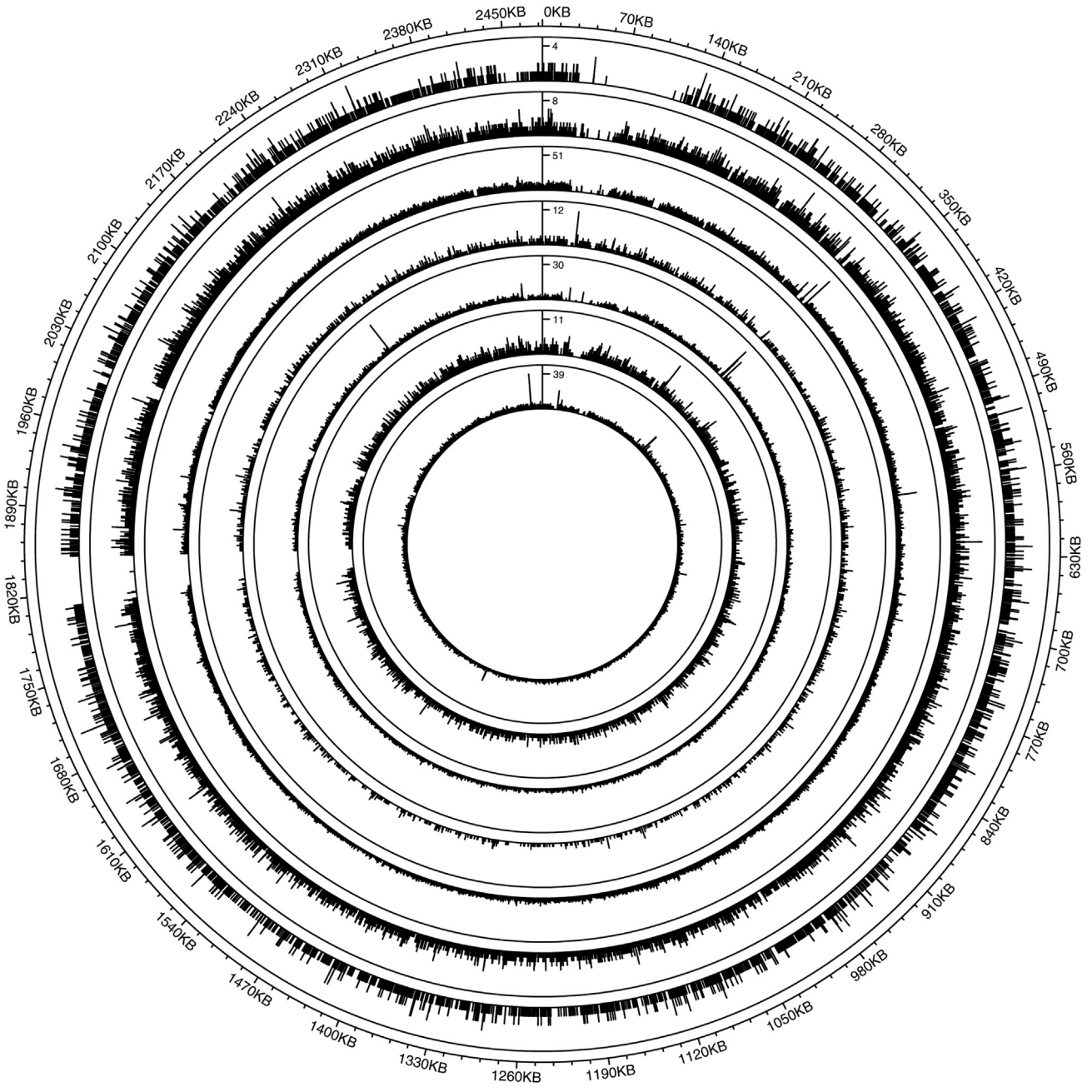

FIGURE 1. Coverage of *S epidermidis* reference genome in 7 culture-negative cases. Outer ring indicates the position in the genome in megabases. Each inner ring represents sequences detected from a culture-negative case.

(Appendix, Supplemental Table 4, Supplemental Figures 3 and 4).

*Higher quantitative bacterial load of non-*S epidermidis *pathogens corresponded to worse outcomes*. Bacterial load was not associated with presenting visual acuities in any groups stratified by culture, WGS status, TTV, or MCV status by Spearman correlation ($P > .05$). The only significant associations between bacterial load and month-1 VA (slope = 0.507; rho = 0.83; $P = 0.011$) and month-3 VA (slope = 0.575; rho = 0.91; $P = .001$) were found in culture-positive, non-*S epidermidis* cases. Similarly, significant correlations between bacterial load and month-1 (slope = 0.485, rho = 0.85; $P = .004$) and month-3 (slope = 0.583, rho = 0.89; $P = .001$) VA were found in non-*S epidermidis*, pathogen-positive cases determined by WGS.

Presence of hypopyon and poor vision at presentation are associated with worse visual outcome. Univariate logistic

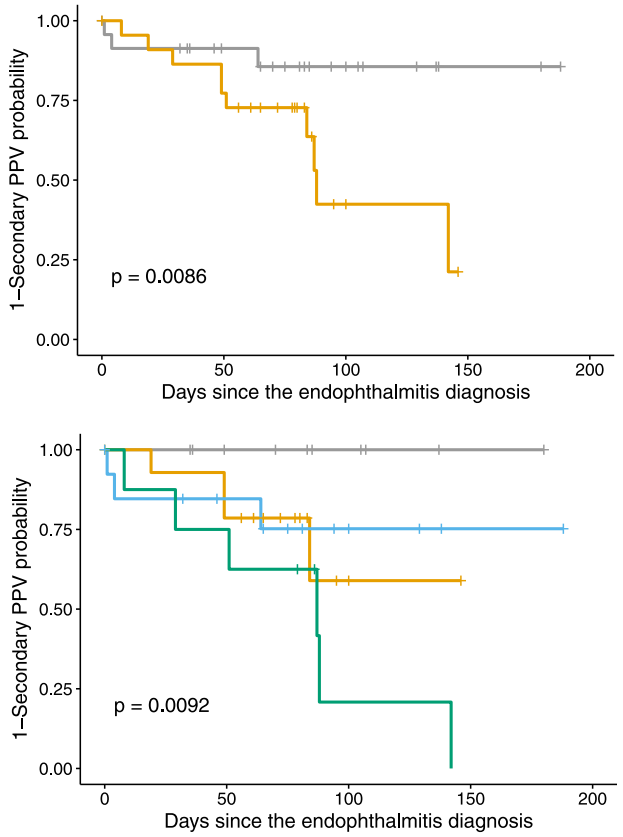

FIGURE 2. (Top) Kaplan-Meier curves for secondary PPV in patients diagnosed with endophthalmitis stratified by TTV-positive (orange) and TTV-negative (gray) status at baseline. (Bottom) Culture and TTV status. Gray indicates both culture- and TTV-negative; orange indicates culture-negative but TTV-positive; blue indicates culture-positive but TTV-negative; Green indicates both culture- and TTV-positive. The x-axis shows days to PPV since the endophthalmitis diagnosis; the y-axis shows the proportion of the cohort who remained free of secondary PPV. PPV = pars plana vitrectomy; TTV = torque teno virus.

regression models on month 1 visual outcome were evaluated in 45 patients who had month 1 follow-up. The only variables that were significantly associated with poor month 1 visual outcome (defined as 20/200 or less) were presenting VA and presence of hypopyon. Per each 1 unit worse logMAR presenting VA, the likelihood of month 1 VA being 20/200 or less was 4.8 times higher ($P = 0.02$) (Supplemental Table 6). Hypopyon at presentation was associated with 12.9 times higher risk of poor month 1 visual outcome (median logMAR VA 1.15 [20/280] vs 0.40 [20/50]; $P = 0.02$) (Supplemental Table 6). When all variables with p-values less than 0.2 (baseline VA, AC cells and flare, and pathogen status) were entered into the multivariate model with month 1 VA as the outcome, no variables showed significant independent association.

On analyses of 43 patients who had month-3 follow-up, the endophthalmitis cases following "other" surgeries (ie, not intravitreal injections or cataract surgery) were associated with worse month-3 VA (median logMAR VA 2.0 [20/2000] in "other" surgery group vs 0.3 [20/40] in either cataract or intravitreal injection group; $P = 0.002$) (Supplemental Table 7). Worse outcomes were associated with noncataract and intravitreal injection cases in multivariate analysis as well when normalized for presenting VA (OR, 43.8; $P = .006$).

Presence of TTV is associated with increased risk of need for secondary vitrectomy. Of 45 patients who had at least 1 month of follow-up, 13 patients underwent secondary vitrectomy (4 culture-negative and 9 culture-positive cases) due to the following complications: retinal detachment (n = 5), visually significant vitreous opacities (n = 5), vitreous hemorrhage (n = 1), epiretinal membrane (n = 1), and miscellaneous (n = 1). However, one of the cases that initially underwent PPV for significant vitreous opacities received a second PPV for retinal detachment.

A total of 10 patients (of 23 [43%]) underwent secondary PPV in the TTV-positive group whereas 3 patients (3 of 24 [13%]) underwent secondary PPV in the TTV-negative group. The odds of having a secondary PPV in patients who had TTV at presentation was 5.2 times greater than the patients who did not have TTV (95% CI 1.07, 34.82; $P = .024$). All other factors such as patient's age and sex, culture and pathogen status, procedure type, baseline hypopyon, and MCV status were not associated with a secondary PPV ($P > .05$). The median TTV load in cases that had a secondary PPV was 1.7×10^4 copies/mL (mean, 12.9×10^4 copies/mL; range, 0-56.3×10^4 copies/mL), which was significantly higher than the median TTV load of 0 (mean, 3.0×10^4 copies/mL; range, 0-38.3×10^4 copies/mL) in cases that did not require a secondary PPV ($P = .023$). A total of 5 of 7 samples (71%) that had TTV greater than 100 copies/μL at baseline ultimately underwent a secondary PPV.

On univariate Kaplan-Meier survival analysis with Fleming-Harrington weighted log-rank test,[13] the following variables were analyzed for potential associations with secondary PPV: presenting VA (worse than hand motion [HM] or better based on EVS data[4]), culture positivity, WGS positivity, TTV presence, and MCV presence. The only variable that was significantly associated with a higher rate of secondary PPV was the presence of TTV ($P = .009$) (Figure 2Top). Interestingly, when the cohort was stratified by culture and TTV status, no dual-culture- or TTV-negative cases underwent secondary PPV, whereas all dual-culture- and TTV-positive cases required secondary PPV ($P = .009$) (Figure 2Bottom).

DISCUSSION

THE CURRENT STUDY IS THE FIRST TO CORRELATE MOLECULAR detection of pathogens through the use of deep sequencing with clinical outcomes in postprocedure endophthalmitis. The study found that most culture-positive samples yielded the same organism on deep sequencing as found in culture but that a significant subset of culture-negative samples showed clear evidence of *S epidermidis* infection, potentially explaining the similar outcomes observed between cases infected with *S epidermidis* and culture-negative cases. It was found that patients with other pathogens generally had worse outcomes and that higher quantitative bacterial load correlated with worse results. Finally, it was found that TTV was found in nearly half of endophthalmitis samples and predicted the need for secondary vitrectomy surgery, particularly in cases that were also culture-positive. Taken together, these results suggest that WGS sequencing and directed PCR can provide improved prognostic information in post-procedural endophthalmitis.

Although standard microbiological culturing remains the gold standard for pathogen detection, molecular testing such as PCR or deep DNA sequencing have been increasingly used in ophthalmology.[15] Although PCR is more sensitive and specific than culture, the main limitation of PCR is the requirement of a priori knowledge of suspected pathogens. However, PCR is an invaluable technique to confirm the pathogen that may have been unexpected or for detecting small organisms such as TTV that may be difficult to detect with "shotgun" sequencing techniques in particular.[16,17]

Deep DNA sequencing such as BRiSK or technologies using Illumina dye, 454 pyrosequencing, or nanopore sequencing have the advantages of not requiring a priori knowledge of pathogens.[18,19] However, high false discovery rate due to paucibacterial samples must be carefully considered when interpreting the deep sequencing results, particularly when using 16S rDNA amplicons.[20,21] To decrease a false discovery rate, all cases that did not have adequate overall DNA reads were excluded first. We aligned all reads to the reference genome of the corresponding pathogen de novo to limit false positives from carry-over amplified DNA contamination. The depth and distribution of genome coverage were evaluated in all cases that were not matching culture results to ensure that the sequencing results were not driven by high repeats of specific sequences.

For example, *S epidermidis* readings were commonly found throughout the samples in this study cohort. To rule out the possibility of false detection, all *S epidermidis* reads from all samples were aligned regardless of the pathogen status to the reference genome. Not surprisingly, the coverage of *S epidermidis* was the highest in the culture- and WGS-positive samples for *S epidermidis*. The coverage of *S epidermidis* in culture-negative but WGS-positive samples was lower than the culture- and WGS-positive cases (reflecting lower bacterial load in these cases) but still showed substantial (60%) genome coverage, suggesting bona fide detection of organismal DNA. The coverage of *S epidermidis* in culture- and WGS-negative samples was minimal, similar to that found in nonendophthalmitis control samples (and perhaps indicative of detection of sparse *S epidermidis* DNA on the ocular surface). Because many reads pertaining to diverse organisms are commonly found in paucibacterial samples,[20] aligning all the sequenced reads to the reference genome is believed to be critical to the conclusion that the organism is present.

In addition to confirming the culture results in most cases, deep sequencing results provided detailed characterization of the pathogens. To illustrate, WGS detected *S epidermidis* as the leading pathogen in all 10 culture-positive samples that grew *S epidermidis*. However, the number of reads of *S epidermidis* varied greatly, ranging from 100 to 1,023,392 (average, 233,774 reads). Despite substantial variation in the infection burden among *S epidermidis* cases, there was no correlation between *S epidermidis* bacterial load and the clinical outcome in this cohort. In contrast, significant negative correlations were found between baseline bacterial loads and month-1 and -3 visual outcome in non-*S epidermidis* cases. Thus, reliable, quantitative, bacterial load information may be a useful biomarker of clinical outcome in non-*S epidermidis* endophthalmitis. Future point-of-service WGS device that would reliably detect the pathogen and its load may be beneficial in initial management of endophthalmitis cases.

Fourteen of the 22 culture-negative samples in the present study did not reveal an organism on WGS analysis. This result is similar to that of the authors' previous study in which culture-negative samples showed no significant bacterial loads by 16S qPCR.[11] The cause of endophthalmitis in these cases was unclear. These cases could represent situations where a small bacterial load was cleared by immune mechanisms prior to sampling; where bacteria were localized in the eye and not sampled by vitreous or aqueous biopsy; or could represent a sterile endophthalmitis or undetected pathogen with similar pathogenic potential as *S epidermidis* (for instance, RNA virus). The finding that outcomes in these WGS-negative samples were similar to those seen with *S epidermidis* and that most culture-negative samples yielding organisms on WGS were *S epidermidis* suggest that WGS and culture-negative samples may reflect very low bacterial load in cases of *S epidermidis* infection. Furthermore, the finding that bacterial load for *S epidermidis* (as measured by genome load) did not influence outcome suggests that the pathogenic mechanisms of this bacteria are not dependent on cooperative or quorum-based pathways.

TTV is a small, nonenveloped, single-stranded, circular DNA virus initially described in a patient with acute post-transfusion hepatitis in 1997.[22] This anellovirus is only 30-

50 nm in diameter and has a very small genome, 3.8 kb in length. TTV is ubiquitous and found in soil, water, air, and many sites of human tissues including serum.[23] TTV has been associated with numerous diseases involving blood, immune system, respiratory system, and cancers but has not been shown to be the cause of any.[24] TTV has been described in vitreous from seasonal hyperacute panuveitis (SHAPU), a rare potentially blinding inflammatory condition that affects primarily children in Nepal.[25] Current studies suggest that TTV is apathogenic but may serve as a marker of immune function and/or opportunistic infections.[26–30] Recent studies have shown significant associations between lower serum level of TTV and higher risks of biopsy-proven rejection in patients with kidney,[31] lung,[32] and liver[33] transplants. Limitations of these studies were small sample size or retrospective nature; however, it is intriguing that high counts of TTV suggested a higher state of immune suppression in these studies. It is possible that a decreased immune state of the host might have allowed a more severe infection leading to worse presentation, outcome, and complications in the present endophthalmitis cohort. Data for the immune status of the present patients were not collected; however, older age was not associated with either TTV or MCV presence in our cohort.

TTV was strongly associated with a higher rate of secondary PPV. TTV was present in 10 of 13 cases that required a secondary PPV, and the odds of having a secondary PPV in patients who had TTV at presentation was 5.2 times greater than in the patients who did not. This was particularly true for the cases that required a secondary PPV for retinal detachment: 100% of these cases had TTV at presentation. Interestingly, even TTV-positive patients who had good visual outcome at month 1 developed retinal detachment or visually significant floaters that led to PPV in our cohort, suggesting that TTV may be useful as an important biomarker for worse outcome. Detection of TTV at presentation could be useful for clinicians to decide how frequently to follow-up on patients who have TTV detected at the time of endophthalmitis diagnosis.

Similar to our results, cases infected with *S epidermidis* have been shown to have better visual outcome than the cases infected with other cultured organisms.[5,34] The present study also adds the important result that no significant differences existed in the visual outcome between *S epidermidis* cases and culture-negative cases. However, even within cases infected with *S epidermidis*, a substantial variation in visual outcome was seen (range, 20/25 to HM), suggesting that additional factors influence outcomes. It is possible that genomic and transcriptional characterization of the individual pathogens and hosts will be helpful in understanding why a large variation exists. Previously, in other infectious conditions, specific mutations in quorum sensing genes or genes involved in biofilm formation have been associated with pathogenicity.[35,36] Future studies to identify specific mutations or molecular variants that influence the virulence of the pathogen through WGS and combining them with the transcriptomic analyses of both pathogen and host will be important in helping to elucidate this relationship.

Several limitations exist in our study. First, there was a limited sample size with an imperfect follow-up rate. Despite these limitations, several variables were found that were associated with visual outcome with statistical significance and a significantly higher hazard rate for secondary PPV in the TTV-positive group compared to the TTV-negative group. The role of TTV is unknown; our results suggest that it may be used as a marker for worse disease severity associated with a worse outcome but do not prove causation. A limited amount of ocular sample was available for molecular testing in this study, which required, in some cases, to choose between tests. The adequate sample amount is dependent on the infectious load of each case, which is difficult to discern at presentation. This study included various types of intraocular samples and patients followed by different retina specialists from 2 different academic centers. Thus, an inherent difference in the quality of samples or data collection, potential impact from the shipping process, and selection bias toward the surgical intervention from different providers cannot be ruled out. In addition, there was no clearly defined protocol for treatment selection, VA testing, or clinical examination scoring. However, this study likely reflects current real-world practice patterns and challenges in diagnosing and treating endophthalmitis patients.

In summary, the present study demonstrates that the status of the culture of molecular pathogen testing (for bacteria and virus) and baseline VA have prognostic significance for clinical outcomes including VA and secondary vitrectomy in endophthalmitis. Molecular studies provide more extensive and sensitive characterization of pathogens and have the potential to allow for improved treatment paradigms. Further development of rapid, point-of-service molecular diagnostics and subsequent prospective randomized controlled clinical trials will allow for testing of new paradigms for risk stratification and individualized treatment for endophthalmitis.

CRediT AUTHORSHIP CONTRIBUTION STATEMENT

CECILIA S. LEE: CONCEPTUALIZATION, METHODOLOGY, Investigation, Writing - original draft. **Bryan Hong:** Investigation, Data curation, Writing - review & editing. **Sundeep K. Kasi:** Investigation, Data curation, Writing - review & editing. **Christopher Aderman:** Investigation, Writing - review & editing. **Katherine E. Talcott:** Investigation, Writing - review & editing. **Murtaza K. Adam:** Investigation, Writing - review & editing. **Bryan Yue:** Investigation, Writing - review & editing. **Lakshmi**

Akileswaran: Investigation, Writing - review & editing. **Kenji Nakamichi:** Methodology, Software, Writing - review & editing. **Yue Wu:** Investigation, Writing - review & editing. **Kasra A. Rezaei:** Investigation, Writing - review & editing. **Lisa C. Olmos de Koo:** Investigation, Writing - review & editing. **Yewlin E. Chee:** Investigation, Writing - review & editing. **Aaron Y. Lee:** Methodology, Software, Writing - review & editing. **Sunir J. Garg:** Conceptualization, Methodology, Investigation, Supervision, Writing - review & editing. **Russell N. Van Gelder:** Conceptualization, Methodology, Investigation, Supervision, Writing - review & editing.

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