The Clinical Value of Diagnostic Vitrectomy (Postprint)

Authors: Di Yu, Ye Junjie

Date: 2018-06-14T00:00:00+00:00

Abstract

Diagnostic vitrectomy is an effective diagnostic method that obtains vitreous, retinal, or choroidal specimens combined with relevant laboratory examination techniques to establish etiology and guide treatment for intraocular inflammatory diseases and malignant tumors of unknown etiology or refractory to conventional therapy. With the advancement of minimally invasive vitreoretinal surgery and laboratory diagnostic techniques, the sensitivity and specificity of diagnostic vitrectomy have been further improved. This article reviews the indications for diagnostic vitrectomy, specimen collection and processing, laboratory detection methods, and advances in minimally invasive vitrectomy.

Full Text

Diagnostic Vitrectomy and Its Clinical Value

Di Yu, Ye Jun-jie
Department of Ophthalmology, Peking Union Medical College Hospital, Chinese Academy of Medical Sciences & Peking Union Medical College, Beijing 100730, China
Corresponding author: Ye Jun-jie, E-mail: yejunjie_xh@163.com

Abstract

Diagnostic vitrectomy is an effective diagnostic procedure that obtains vitreous, retinal, or choroidal specimens to establish etiological diagnoses for intraocular inflammatory diseases and malignant tumors of unclear origin or those unresponsive to conventional treatments, thereby guiding subsequent management. The advent of minimally invasive vitreoretinal surgery and advances in laboratory diagnostic technologies have further enhanced the sensitivity and specificity of diagnostic vitrectomy. This review summarizes the indications for diagnostic

vitrectomy, specimen collection and processing protocols, laboratory detection methods, and recent developments in minimally invasive vitrectomy techniques.

Keywords: Diagnostic vitrectomy; Minimally invasive; Laboratory diagnostics

---

Introduction

Diagnostic vitrectomy involves obtaining vitreous, retinal, or choroidal specimens combined with laboratory testing to establish etiological diagnoses for various intraocular inflammatory diseases and malignant tumors, guiding further treatment. Since the introduction of sutureless 25-gauge and 23-gauge vitrectomy systems in the early 21st century, vitreoretinal surgery has entered a minimally invasive era. Coupled with rapid developments in laboratory diagnostic technologies, the safety and efficacy of diagnostic vitrectomy have improved considerably, expanding its clinical applications in ophthalmology.

1. Clinical Applications of Diagnostic Vitrectomy

Diagnostic vitrectomy plays a crucial role in diagnosing intraocular inflammatory diseases and malignant tumors of unknown etiology or those refractory to treatment, while simultaneously offering therapeutic benefits.

1.1 Uveitis of Unknown Etiology or Refractory to Treatment Establishing an etiological diagnosis is essential for treatment selection and prognosis in uveitis. While most cases can be diagnosed through detailed history, comprehensive ocular examination, and ancillary laboratory tests, tissue biopsy becomes necessary when uveitis presents with atypical clinical features or fails to respond to conventional therapy. Diagnostic vitrectomy demonstrates high sensitivity and specificity for early pathogen identification. Lin et al. reported a diagnostic yield of 59.7% in 65 patients with undifferentiated uveitis who underwent diagnostic vitrectomy. Literature indicates that diagnostic vitrectomy yields diagnostic rates ranging from 12.4% to 64.3%, with infectious uveitis diagnosis rates of 27.9% to 77.1%. Bacterial or fungal uveitis is typically diagnosed through Gram staining and culture of vitreous specimens, with diagnostic rates of approximately 16.7% to 96%. William et al. performed 23-gauge diagnostic vitrectomy in 15 patients, confirming endogenous fungal endophthalmitis in 12 cases (75%). Toxoplasma infection can be detected through PCR combined with Goldmann-Witmer coefficient analysis. Westeneng et al. performed diagnostic vitrectomy in 54 patients, diagnosing toxoplasmosis in 16 cases (30%), herpes simplex virus in 13 cases (24%), and varicella-zoster virus in 16 cases (30%).

1.2 Primary Intraocular Lymphoma Primary intraocular lymphoma (PIOL) often presents as a masquerade syndrome with high misdiagnosis rates, leading to delayed treatment, loss of vision-saving opportunities, and life-threatening complications. Histopathology remains the gold standard for

PIOL diagnosis, and diagnostic vitrectomy is the most effective method for obtaining vitreous fluid while simultaneously clearing opacified vitreous to improve visual acuity. Yeh et al. performed 25-gauge diagnostic vitrectomy in 12 clinically suspected PIOL cases, confirming B-cell or T-cell lymphoma in 11 cases through cytopathological analysis, flow cytometry, and molecular biological testing. Following targeted treatment and a mean follow-up of 37 weeks, visual acuity stabilized or improved in 11 eyes, with average vision increasing from 20/95 to 20/66.

1.3 Therapeutic Role of Diagnostic Vitrectomy Diagnostic vitrectomy reduces vitreous opacification and removes inflammatory mediators from the vitreous cavity, including cytokines, interleukins, and activated lymphocytes. When combined with intraocular drug injection, it significantly attenuates inflammatory responses. Oahalou et al. reported that minimally invasive diagnostic vitrectomy improves visual outcomes and reduces systemic immunosuppressive therapy requirements. In their study of 18 patients, mean visual acuity improved from 20/200 to 20/80 postoperatively, with 8 patients (44%) discontinuing immunosuppressive therapy and 3 patients (16.7%) reducing dosage. They proposed that diagnostic vitrectomy provides equivalent benefits to therapeutic vitrectomy. Sato further demonstrated visual acuity improvements in both infectious and non-infectious uveitis following diagnostic vitrectomy. Pakdel et al. observed regression of subretinal lymphoma lesions after diagnostic vitrectomy, attributing this to the activation of abundant CD4+ T-cells during vitrectomy, which inhibits B-cell proliferation in diffuse large B-cell lymphoma, the most common PIOL subtype.

2. Specimen Collection and Processing for Diagnostic Vitrectomy

Diagnostic vitrectomy encompasses vitreous, retinal, and choroidal biopsies, with vitreous biopsy being most common. Preoperative selection of appropriate tests based on clinical data, meticulous intraoperative technique to obtain adequate samples, and prompt postoperative processing to ensure specimen integrity are essential for maximizing diagnostic yield.

2.1 Vitreous Biopsy Specimen Acquisition Technique: Vitreous specimens can be obtained during vitrectomy. The infusion line is initially closed, and a 3 mL syringe is connected to the vitrectomy cutter aspiration tubing. The cutter is positioned in the mid-peripheral vitreous for automated cutting with manual aspiration to obtain undiluted vitreous specimens. When the globe begins to soften, the infusion is opened, and a 5 mL or 10 mL syringe is connected to continue manual aspiration for diluted vitreous specimens. Finally, the vitrectomy machine collection cassette yields additional specimens.

Specimen Volume and Test Selection: Vitreous sample volume is typically limited by hypotension complications, with 1.5–2.0 mL of undiluted vitreous usually obtained. Mercado et al. proposed using perfluorocarbon instead

of balanced salt solution to obtain 2.24 mL of undiluted vitreous. Zhang et al. suggested injecting gas through the infusion line during aspiration to obtain 3 mL specimens. Undiluted samples are processed via cytocentrifugation for cytopathology and immunohistochemistry, with supernatant used for cytokine and antibody analysis, while molecular biological analysis requires undiluted vitreous. Typically, 3–4 mL of diluted vitreous is obtained for flow cytometry and microbial culture, which can also undergo cytological analysis after centrifugation. The 50 mL specimen from the collection cassette can be used for microbial culture and sensitivity testing.

Specimen Handling and Yield Optimization: Vitreous specimens must be promptly delivered to the laboratory as cells degenerate rapidly. In PIOL cases, vitreous specimens undergo degeneration within minutes. Ranty et al. demonstrated that immediate placement of undiluted vitreous in collection tubes containing RPMI-1640 medium, fetal bovine serum, and gentamicin effectively preserves cellular integrity, maintaining high diagnostic accuracy even when cytological analysis is performed one hour after collection. Ethanol fixation should be avoided in suspected PIOL cases as it causes lymphocyte degeneration. Specimens for immunoglobulin titer analysis require freezing. Additionally, glucocorticoids cause lymphocyte degeneration, so systemic and topical steroids should be discontinued in suspected PIOL patients to enhance cellular viability and quantity. Studies indicate that mid-peripheral vitreous contains more cellular components than central vitreous, yielding higher positive rates for cytopathological detection. When specimen quality is poor and laboratory detection rates are low, repeat diagnostic vitrectomy may be considered, though cell numbers are reduced after initial vitrectomy, necessitating retinal or choroidal biopsy. Currently, no single laboratory method achieves complete sensitivity, making combined testing approaches essential for improving diagnostic yield.

2.2 Retinal and Choroidal Biopsy For fundus diseases involving the retina and choroid, targeted biopsies can be performed. Lesions anterior to the equator can be accessed via external approaches, while posterior lesions require transretinal fine-needle aspiration or vitrectomy-based sampling. External approaches are limited by choroidal hemorrhage and tumor seeding risks, whereas vitrectomy techniques have fewer complications and are preferred for retinal and choroidal biopsies.

Diagnostic vitrectomy typically targets the junction between actively replicating lesions and normal retina. If the retina is attached, three rows of laser photocoagulation are applied at the biopsy site. After retinotomy, saline injection creates a detachment, and intraocular scissors excise the lesion while intraocular pressure elevation to 70–90 mmHg minimizes bleeding. Specimens are retrieved with intraocular forceps. In cases with pre-existing retinal detachment, tissue is excised from the selected area, followed by fluid-air exchange, endolaser photocoagulation, and gas or silicone oil tamponade. Retinal and choroidal specimens should be immediately placed in glutaraldehyde for electron microscopy or for-

malin for light microscopy, with coordination with pathology laboratory staff to determine optimal processing timing for histopathological evaluation. Seregard et al. reported a 95% detection rate (41/43) with high sensitivity (0.97) and specificity (1.00) for choroidal biopsy via 25-gauge vitrectomy in clinically indeterminate choroidal tumors.

3. Common Detection Methods for Diagnostic Vitrectomy Specimens

3.1 Cytopathological Detection Cytopathology is the gold standard for diagnosing intraocular malignancies, typically using undiluted vitreous fluid. Margolis et al. reported 66.7% sensitivity for cytopathological detection of intraocular malignancies, with higher sensitivity for PIOL (83.3%) than metastatic tumors (33.3%). Raparia et al. reported 87.5% sensitivity (14/16) for PIOL diagnosis. When cytopathology is negative but PIOL remains highly suspected, immunohistochemistry, flow cytometry, and molecular biology techniques can provide definitive diagnosis. Adrienne et al. identified sarcoid uveitis in 5.3% (8/150) of eyes through cytopathological exclusion of tumor- and infection-related uveitis combined with clinical data.

3.2 Immunohistochemistry and Flow Cytometry Immunohistochemistry identifies cell types through specific antibody binding to cell surface markers, providing qualitative, localized, and quantitative data, typically using undiluted vitreous. When sample volume is limited, diluted vitreous can be analyzed via flow cytometry to simultaneously detect multiple cell surface markers and record cell counts. B-cell lymphoma exhibits restricted and chain expression, with : ratios 3 or 0.6 serving as sensitive markers. Davis et al. reported 80% sensitivity for : ratio (3 or 0.6) in PIOL diagnosis, while CD22 and CD20 showed lower sensitivity (50% and 30%) but high specificity (94% and 89%). CD4:CD8 ratio 4 demonstrates high sensitivity (64%) and specificity (85%) for infectious uveitis. Flow cytometry achieves 83.3% sensitivity for PIOL diagnosis but lower sensitivity for chronic idiopathic uveitis.

3.3 Cytokine Detection Cytokine analysis primarily measures IL-10, IL-6, and IL-2 concentrations in diluted vitreous. Tumor B-cells secrete abundant IL-10, while inflammatory B-cells produce IL-6. Under normal conditions, aqueous humor and vitreous lack IL-10 and IL-6 expression. Therefore, measuring IL-10 levels and the IL-10:IL-6 ratio facilitates B-cell lymphoma diagnosis. Studies show that an IL-10:IL-6 ratio >1 strongly suggests B-cell lymphoma, with 81.8% sensitivity and 100% specificity. Fisson et al. analyzed interferon- , tumor necrosis factor- , IL-6, and IL-10 in aqueous and vitreous specimens from PIOL and uveitis patients, demonstrating that IL-10:IL-6 and IL-10:IFN- ratios can differentiate PIOL from uveitis.

3.4 Molecular Biology Techniques Polymerase chain reaction (PCR) enables microbiological diagnosis by amplifying bacterial 16S rRNA and fungal

18S rRNA genes, complementing conventional microbial culture for bacterial and fungal detection. Misstate et al. combined PCR with Goldmann-Witmer coefficient analysis [vitreous-specific antibody IgG/vitreous total IgG]/[plasma-specific antibody IgG/plasma total IgG] to improve virological detection rates. PCR-based immunoglobulin heavy chain (IgH) and T-cell receptor (TCR) gene rearrangement analysis aids B-cell and T-cell lymphoma diagnosis. Yeh et al. achieved the highest detection rate (80%, 8/10) through gene rearrangement analysis in 12 suspected PIOL cases, compared to cytokine analysis (37.5%, 3/8) and flow cytometry (33.3%, 4/12). Harper et al. reported high specificity (97.4%) and sensitivity (80.9%) for PCR detection of retinochoroiditis in 133 patients undergoing diagnostic vitrectomy.

3.5 Microbial Culture and Sensitivity Testing Conventional bacterial and fungal endophthalmitis detection includes Gram staining and microbial culture, typically using diluted vitreous fluid, with detection rates of 16.7% to 96%. Joseph reported higher PCR detection rates (66%) compared to microbial culture (34%) in 64 suspected bacterial endophthalmitis cases. Arvanitis et al. demonstrated that molecular biology techniques facilitate rapid fungal endophthalmitis diagnosis with 43% to 100% sensitivity. Liu et al. reported higher sensitivity for histopathological fungal detection (70%) versus conventional microbial culture (40%), recommending combined use of both methods.

4. Minimally Invasive Diagnostic Vitrectomy

Machemer et al. first applied pars plana vitrectomy in 1971. To reduce surgical trauma and improve efficiency, diagnostic vitrectomy has evolved toward minimally invasive techniques, with recent reports documenting clinical application of even smaller 27-gauge systems.

4.1 Advantages of Minimally Invasive Diagnostic Vitrectomy Minimally invasive diagnostic vitrectomy clears opaque intraocular media for better visualization, obtains adequate vitreous and retinochoroidal specimens, and enhances diagnostic rates through comprehensive laboratory testing. Yeh et al. confirmed PIOL in 11 of 12 suspected cases (92%) using 25-gauge diagnostic vitrectomy. Kanavi et al. diagnosed PIOL in 15 of 18 eyes (83%) undergoing 25-gauge diagnostic vitrectomy. Compared to conventional 20-gauge vitrectomy, minimally invasive techniques eliminate conjunctival incision and sclerotomy suturing, simplifying procedures and reducing operative time. Smaller incisions promote faster healing, with unsutured sclerotomies healing more naturally, minimizing conjunctival scarring and corneal astigmatism while improving postoperative comfort.

4.2 Limitations of Minimally Invasive Diagnostic Vitrectomy Complications of minimally invasive diagnostic vitrectomy are relatively uncommon, including retinal detachment, complicated cataract, transient postoperative hypotony, and endophthalmitis. William et al. reported retinal detachment in

42% (8/19), complicated cataract in 32% (6/19), and transient hypotony in 5% (1/19) after 23-gauge diagnostic vitrectomy for fungal endophthalmitis. Oshima et al. found similarly low endophthalmitis rates after 25-gauge and 20-gauge diagnostic vitrectomy without significant differences. Hu et al. confirmed these findings, attributing postoperative endophthalmitis to unsutured sclerotomies and transient hypotony facilitating entry of extraocular fluid and microorganisms. Recent studies have identified tumor seeding risks after diagnostic vitrectomy for suspected malignancies. Kung et al. reported a case of choroidal metastasis from breast ductal adenocarcinoma that presented as bullous retinal detachment. After diagnostic vitrectomy with inconclusive cytopathology, a tumor-like mass appeared at the sclerotomy site six months later, suggesting tumor cell dissemination from the diagnostic procedure.

Conclusion

The evolution of minimally invasive vitreoretinal surgery and continuous improvements in laboratory diagnostic technologies have endowed diagnostic vitrectomy with high sensitivity and specificity for disease diagnosis, enabling early etiological identification and effective treatment planning. This provides clinicians with powerful diagnostic tools and benefits patients considerably. However, minimally invasive diagnostic vitrectomy has certain limitations. With further advances in minimally invasive techniques, expanded indications, and reduced postoperative complications, the application prospects for diagnostic vitrectomy in ophthalmic disease diagnosis will become increasingly broad.

References

[1] Oahalou A, Schellekens PA, de Groot-Mijnes JD, et al. Diagnostic pars plana vitrectomy and aqueous analysis in patients with uveitis of unknown cause[J]. Retina, 2014, 34(1): 108-114.

[2] Lin HY, Dai RP, et al. Analysis of diagnostic results from vitrectomy specimens in 65 patients with undifferentiated uveitis[J]. Chinese Journal of Ocular Fundus Diseases, 2017, 7(33): 404-406.

[3] Jeroudi A, Yeh S. Diagnostic Vitrectomy for Infectious Uveitis[J]. Int Ophthalmol Clin, 2014, 54(2): 173-197.

[4] Bispo PJ, Melo GB, d' Azevedo PA, et al. Culture proven bacterial endophthalmitis: a 6-year review[J]. Arq Bras Oftalmol, 2008, 71(5): 617-622.

[5] William A, Spitzer MS, Deuter C, et al. Outcomes of Primary Transconjunctival 23-Gauge Vitrectomy in the Diagnosis and Treatment of Presumed Endogenous Fungal Endophthalmitis[J]. Ocul Immunol Inflamm, 2016, 30: 1-7.

[6] Westeneng AC, Rothova A, de Boer JH, et al. Infectious uveitis in immunocompromised patients and the diagnostic value of polymerase chain reaction and Goldmann-Witmer coefficient aqueous analysis[J]. Am J Ophthalmol, 2007, 144(5): 781-785.

[7] Hochberg FH, Miller DC. Primary central nervous system lymphoma[J]. J Neurosurg, 1988, 68(6): 835-853.

[8] Yeh S, Weichel ED, Faia LJ, et al. 25-Gauge transconjunctival sutureless vitrectomy for the diagnosis of intraocular lymphoma[J]. Br J Ophthalmol, 2010, 94(5): 633-638.

[9] Sato T, Kinoshita R, Taguchi M, et al. Assessment of diagnostic and therapeutic vitrectomy for vitreous opacity associated with uveitis with various etiology[J]. Medicine, 2018, 97(2): e9491.

[10] Pakdel A, Mammo Z, et al. Regression of subretinal lymphoma after diagnostic vitrectomy[J]. JAMA Ophthalmol, 2017, 135(5): 503-505.

[11] Quiroz-Mercado H, Rivera-Sempertegui J, Macky TA, et al. Performing vitreous biopsy with perfluorocarbon-perfused vitrectomy[J]. Am J Ophthalmol, 2005, 140(6): 1161-1163.

[12] Zhang L, Yang X, Zheng Q, et al. Binocular indirect ophthalmoscope-assisted gas-perfused pars plana vitrectomy: a novel technique for vitreous sample acquisition[J]. Medicine, 2016, 95(49): e5503.

[13] Margolis R. Diagnostic vitrectomy for the diagnosis and management of posterior uveitis of unknown etiology[J]. Curr Opin Ophthalmol, 2008, 19(3): 218-224.

[14] Ranty ML, Laurent C, Aziza J, et al. Improving cytological diagnosis of intraocular lymphoma from vitreous fluid[J]. Histopathology, 2015, 67(1): 48-61.

[15] Errera MH, Goldschmidt P, Batellier L, et al. Real-time polymerase chain reaction and intraocular antibody production for the diagnosis of viral versus toxoplasmic infectious posterior uveitis[J]. Graefes Arch Clin Exp Ophthalmol, 2011, 249(12): 1837-1846.

[16] Mudhar HS, Sheard R, et al. Diagnostic cellular yield is superior with full pars plana vitrectomy compared with core vitreous biopsy[J]. Eye, 2013, 27(1): 50-55.

[17] Seregard S, All-Ericsson S, Hjelmqvist L, et al. Diagnostic incisional biopsies in clinically indeterminate choroidal tumours[J]. Eye, 2013, 27(2): 115-118.

[18] Coupland SE, Heimann H, Bechrakis NE. Primary intraocular lymphoma: a review of the clinical, histopathological and molecular biological features[J]. Graefes Arch Clin Exp Ophthalmol, 2004, 242(11): 901-913.

[19] Raparia K, Chang CC, Chevez-Barrios P, et al. Intraocular Lymphoma: Diagnostic Approach and Immunophenotypic Findings in Vitrectomy Specimens[J]. Arch Pathol Lab Med, 2009, 133(8): 1233-1237.

[20] Scott AW, Mruthyunjaya P, McCallum RM, et al. Diagnostic yield of vitreous biopsy in presumed sarcoidosis-related posterior segment inflammation[J].

Graefes Arch Clin Exp Ophthalmol, 2012, 250(9): 1379-1385.

[21] Li M, Ye J. Research progress in primary intraocular lymphoma[J]. Chin J Ophthalmol, 2015, 51: 795-800.

[22] Davis JL, Miller DM, Ruiz P. Diagnostic testing of vitrectomy specimens[J]. Am J Ophthalmol, 2005, 140(5): 822-829.

[23] Sugita S, Takase H, Sugamoto Y, et al. Diagnosis of intraocular lymphoma by polymerase chain reaction analysis and cytokine profiling of the vitreous fluid[J]. Jpn J Ophthalmol, 2009, 53(3): 209-214.

[24] Fisson S, Ouskrim H, Touitou V, et al. Cytokine profile in human eyes: contribution of a new cytokine combination for differential diagnosis between intraocular lymphoma or uveitis[J]. PLoS One, 2013, 8(2): e52385.

[25] Kitiratschky VB, Deuter C, Beck R, et al. Relationship between suspected reasons of intraocular inflammation and the results of diagnostic vitrectomy: an observational study[J]. Ocul Immunol Inflamm, 2015, 23(1): 59-66.

[26] Misstate T, Tielemans D, Bromberg JE, et al. Multicolor flow cytometric immunophenotyping is a valuable tool for detection of intraocular lymphoma[J]. Ophthalmology, 2013, 120(5): 991-996.

[27] Harper TW, Miller D, Schiffman JC, et al. Polymerase chain reaction analysis of aqueous and vitreous specimens in the diagnosis of posterior segment infectious uveitis[J]. Am J Ophthalmol, 2009, 147(1): 140-147.

[28] Joseph CR, Lalitha P, Sivaraman KR, et al. Real-time polymerase chain reaction in the diagnosis of acute postoperative endophthalmitis[J]. Am J Ophthalmol, 2012, 153(6): 1161-1163.

[29] Arvanitis M, Anagnostou T, Fuchs BB, et al. Molecular and nonmolecular diagnostic methods for invasive fungal infections[J]. Clin Microbiol Rev, 2014, 27(3): 490-526.

[30] Liu K, Fang F, Li H. Reliability of vitreous histological detection of pathogenic fungi in the diagnosis of fungal endophthalmitis[J]. Eye, 2015, 29(3): 424-427.

[31] Machemer R, Parel JM, Norton EW, et al. Vitrectomy: a pars plana approach[J]. Trans Am Acad Ophthalmol Otolaryngol, 1971, 75: 813-820.

[32] O' Malley C, Heintz RM Sr. Vitrectomy with an alternative instrument system[J]. Ann Ophthalmol, 1975, 7: 585-588, 591-594.

[33] Kanavi MR, Soheilian M, Hosseini SB, et al. 25-gauge transconjunctival diagnostic vitrectomy in suspected cases of intraocular lymphoma: a case series and review of the literature[J]. Int J Ophthalmol, 2014, 7(3): 577-580.

[34] Oshima Y, Kadonosono K, Yamaji H, et al. Multicenter survey with a systematic overview of acute-onset endophthalmitis after transconjunctival microincision vitrectomy surgery[J]. Am J Ophthalmol, 2010, 150(5): 716-725.

[35] Hu AYH, Bourges JL, Shah SP, et al. Endophthalmitis after pars plana vitrectomy: a 20- and 25-gauge comparison[J]. Ophthalmology, 2009, 116(7): 1360-1365.

[36] Kung YH, Wu TT, Lin CS. Tumor seeding after diagnostic vitrectomy for choroidal metastasis in breast cancer[J]. J Chin Med Assoc, 2012, 75(9): 483-486.

Note: Figure translations are in progress. See original paper for figures.