Retinal Microangiopathy in Human Immunodeficiency Virus Infection is Related to Higher Human Immunodeficiency Virus-1 Load in Plasma

Hansjakob Furrer, MD,[1] Antonio Barloggio, MD,[2] Martin Egger, MD,[1] and the Swiss HIV Cohort Study, Justus G. Garweg, MD[2]

Purpose: To evaluate the prevalence of retinal microangiopathy in human immunodeficiency virus (HIV)-1–infected patients and its association with virologic, immunologic, and sociodemographic parameters.

Design: Single-center cross-sectional study.

Participants: One hundred eighty-eight HIV-1-positive individuals from a single outpatient clinic.

Methods: Human immunodeficiency virus-positive patients were screened for signs of HIV-associated retinal angiopathy. Plasma HIV-1 RNA and CD4-positive cell counts were monitored within 3 months of the ophthalmologic assessment. The absence or presence of angiopathy or of opportunistic viral retinitis was then correlated to data respecting CD4-positive cell count, plasma viral load of HIV-1, and sociodemographic parameters.

Main Outcome Measures: Association between CD4-positive cell count, HIV-1 plasma viral load, sociodemographic parameters, and the manifestation of retinal microangiopathy.

Results: At the baseline consultation, 130 (69%) patients exhibited no retinal pathologic features, 45 (24%) manifested retinal angiopathy, and 13 (7%) had opportunistic viral retinitis. In univariate analysis, retinal angiopathy was associated with lower CD4-positive cell count and higher HIV-1 plasma viral load. In a multivariate logistic model, the presence of retinal microangiopathy was associated with higher age ($P = 0.02$) and higher viral load of HIV-1 ($P < 0.005$), but not with lower CD4 cell counts ($P > 0.05$).

Conclusions: Human immunodeficiency virus-associated retinal microangiopathy is likely a multifactorial condition. Its presence is associated with higher age and higher replication of HIV-1 as measured by plasma HIV-1 RNA levels. In contrast to opportunistic infectious retinitis, the degree of immunodeficiency does not seem to be independently correlated with retinal angiopathy. *Ophthalmology 2003;110:432–436 © 2003 by the American Academy of Ophthalmology.*

Although the clinical picture of ocular involvement in human immunodeficiency virus (HIV) infection has changed dramatically since the advent of potent antiretroviral therapy, microangiopathy is still the most common retinal manifestation. During the early stages of HIV infection, retinal microangiopathy characterized by cotton-wool spots occasionally associated with intraretinal hemorrhage, and other abnormalities of smaller vessels are seen only rarely. However, its incidence increases with time, and up to 50% of patients with acquired immune deficiency syndrome manifest such changes.[1–5] Cotton-wool spots are also observed in association with several other systemic vascular conditions, such as diabetes, hypertension, and lupus erythematosus.[6–8] In contrast to these diseases, however, the changes seen in HIV infection, at least in our experience, occur preferentially at the posterior pole of the eye and less around the optic disc.[5] Not surprisingly, fluorescein angiography often reveals areas of nonperfusion, microaneurysms, and teleangiectasia. The cotton-wool spots disappear and intraretinal hemorrhage subsides after a few weeks. This helps distinguish these conditions from opportunistic viral retinopathies. Moreover, they do not usually become symptomatic, the observable visual function remaining stable.[9] If symptomatic, however, the clinical condition is barely distinguishable from infectious retinopathies.

The cause of HIV-associated retinal microangiopathy is poorly understood. Cotton-wool spots represent axoplasmal swelling that is most likely caused by focal ischemia. The mechanisms underlying this ischemia are not known with

Originally received: October 2, 2001.
Accepted: October 1, 2002. Manuscript no. 210836.

[1] Division of Infectious Diseases, University of Bern, Bern, Switzerland.

[2] Department of Ophthalmology, University of Bern, Bern, Switzerland.

Supported in part by the Federal Office of Health, Bern, Switzerland (grant no.: 95-7225) and by the Swiss National Science Foundation, Bern, Switzerland (grant no.: 3345-062041) in the framework of the Swiss HIV Cohort Study.

Correspondence to Hansjakob Furrer, MD, Division of Infectious Diseases, University Hospital Bern, Inselspital PKT2 B, CH - 3010 Bern, Switzerland. E-mail: hansjakob.furrer@insel.ch

ISSN 0161-6420/03/$–see front matter
doi:10.1016/S0161-6420(02)01750-5

certainty. It has been postulated that immune complexes are first deposited within the vascular endothelium, leading to dysfunction of plasma cells,[10] infection of endothelial cells by HIV—either alone[11,12] or in combination with other viruses[13]—and an increase in fibrinogen levels, which promotes focal clotting.[14] As a consequence, capillaries become occluded.[15] In analogy to angiopathies of noninfectious origin, pericytes are lost,[11] thus paving the way for the formation of teleangiectasia and microaneurysms.[2]

As is known from autopsy studies, these structural changes cause vascular leakage of particles up to 200 nm in diameter.[16] It has been concluded that breakdown of the uveovascular barrier is thus sufficiently gross to permit the spread of viral particles (such as cytomegalovirus) into the retina and therein to induce acute retinitis. Nevertheless, a correlation between the development of opportunistic viral infections and the clinical manifestation of angiopathy has not been demonstrated thus far. According to our own angiographic observations, this circumstance may be accounted for by the uveovascular barrier becoming compromised at a different and probably much earlier stage than that at which retinal angiopathy becomes clinically manifest. Conversely, quantification of uveovascular barrier breakdown has failed to disclose a correlation between anterior chamber flare and severity of retinal angiopathy.[17]

Several groups of investigators have demonstrated an association between the development and progression of angiopathy and the presence of laboratory markers of HIV disease.[5,18,19] It was the aim of the present study to determine whether an association exists between the two most important virologic and immunologic parameters of HIV infection, namely CD4-positive lymphocyte counts (CD4 count) and viral load of HIV-1 as measured by plasma HIV-RNA and by the presence of HIV-associated retinal microangiopathy.

Patients and Methods

Patients

Consecutive HIV-1–infected patients referred to the ophthalmology department from the local HIV outpatient clinic between January 1996 and March 2000 were included in this study if they gave informed consent for this study and if they had a measurement of plasma HIV-RNA and CD4 count available within 3 months of the ophthalmologic assessment. One hundred eighty-eight patients representing 24% of the patients seen at the HIV clinic during the study period were included. At each consultation, information respecting current medication, past or active opportunistic infections, CD4 count, and plasma HIV-RNA was recorded. All investigations, including regular eye examinations, were performed according to the routine examination protocols offered to all participants of the Swiss HIV Cohort Study[20] on a voluntary basis, independent of their contribution to this study. No additional efforts were demanded from the participants. The study had been acknowledged by the local ethics committee and legal institutions.

Laboratory Markers of Human Immunodeficiency Virus Infection

CD4 counts were measured using flow-cytometric assays, and plasma HIV-RNA levels were measured using the Roche Amplicor assay (Roche Diagnostics, Switzerland) with a lower detection limit of approximately 200 copies/ml. An ultrasensitive modification of the assay with a limit of detection of 2 to 40 copies/ml was introduced during 1998. Only data obtained from the first visit of each patient were used for the prevalence analysis.

Ophthalmologic Assessment

On the basis of binocular funduscopy findings at the baseline consultation, subjects were classified according to whether they had retinal microangiopathy and whether they had signs of active or past opportunistic viral retinitis. For this assessment, a slit lamp and Volk Super Field lens Volk Optical, Mentor, Ohio were used, as well as an indirect stereo funduscope and a 22-diopters lens.

Human immunodeficiency virus-associated retinal angiopathy was defined in particular by the clinically detectable presence of retinal cotton-wool spots; intraretinal hemorrhages or clinically detectable microvascular occlusions in association with marked venous dilatation were also included, after vasculopathies of other (i.e., diabetic, hypertensive, and autoimmune) origin had been excluded. In all cases with infectious retinopathy, cytomegalovirus infection had been diagnosed empirically by one observer (JGG) and independently confirmed by amplification of viral DNA from aqueous humor drawn at the time of clinical diagnosis.[21] In cases of doubt, 50° fundus photographs were performed in every instance as a basis for decision but did not have to be used in any instance.

Statistical Methods

For statistical analysis, the database of the ophthalmologic assessment was merged with the database of the Swiss HIV Cohort Study that includes the most important sociodemographic data of each patient.

We used the Wilcoxon rank sum test for comparison of numerical data and the chi-square test for categorical data. Associations between the prevalence of microangiopathy and clinical, laboratory, and sociodemographic data were assessed by univariate logistic regression. A multivariate logistic regression model provided the adjusted odds ratio for the presence of microangiopathy and retinitis for each variable.

A two-sided P value less than 0.05 was considered significant. Stata 7 statistical software (College Station, TX) was used.

Results

The characteristics of the 188 patients enrolled are shown in Table 1. Forty-five patients (23.9%; 95% confidence interval, 18.0%–30.6%) manifested microangiopathy and 16 patients (8.5%; 95% confidence interval, 4.9%–13.4%) had viral retinitis. The history, clinical presentation, and response to treatment of the latter were consistent with retinitis caused by cytomegalovirus. In patients with viral retinitis, retinal microangiopathy was present in 10 of 16 instances (63%).

The median CD4-positive cell count in the studied patient group was 131 cells/μl and 117 patients (62%) had CD4 counts of fewer than 200 cells/μl. Median plasma HIV-RNA was 4.5 $\log_{10}$ copies/ml and 37 participants (20%) had an HIV load of fewer than 400 copies/ml. Thus, the studied population was mainly in a state

Table 1. Characteristics of the 188 Patients Enrolled in the Study of Retinal Microangiopathy in Human Immunodeficiency Virus Infection

	All (n = 188)	With Angiopathy (n = 45)	Without Angiopathy (n = 143)
Females, n (%)	59 (31.4%)	18 (40.0%)	41 (28.7%)
Age (yrs), median (IQR)	36 (32–42)	37.5 (31.8–42.6)	35.6 (32.1–41.6)
Transmission risk, n (%)			
Homosexual intercourse between males	57 (30.3%)	13 (28.9%)	44 (30.8)
Heterosexual intercourse	59 (31.4%)	14 (31.1%)	45 (31.5%)
Intravenous drug use	57 (30.3%)	16 (35.6%)	41 (28.7%)
Other	15 (8.0%)	2 (4.4%)	13 (9.1%)
CDC clinical stage, n (%)			
A	49 (26.1%)	8 (17.8%)	41 (28.7%)
B	59 (31.4%)	13 (28.9%)	46 (32.2%)
C	80 (42.6%)	24 (53.3%)	56 (39.2%)
CD4-positive cell count/μl, median (IQR)	131 (47–304)	60 (12–190)*	158 (59–342)*
CD4-positive cell count (percentage of total lymphocyte count), median (IQR)	12 (5–19)	6 (3–12)*	14 (7–20)*
Log_{10} of plasma HIV RNA (copies/ml), median (IQR)	4.5 (2.9–5.3)	5.3 (4.6–5.7)*	4.2 (2.8–5.1)*
Antiretroviral therapy			
None	51 (27.1%)	14 (31.1%)	37 (25.9%)
One drug	6 (3.2%)	2 (4.4%)	4 (2.8%)
Two drugs	43 (22.9%)	12 (26.7%)	31 (21.7%)
Three or more drugs	88 (46.8%)	17 (37.8%)	71 (49.7%)

CDC = Centers for Disease Control and Prevention; HIV = human immunodeficiency virus; IQR = interquartile range.
Numbers (percentages) or median values (IQR) are presented. Significant differences between the two groups of patients with or without angiopathy are indicated by an asterisk (*).
*$P < 0.01$, Wilcoxon rank sum test.

of significant immunodeficiency and only a minority were treated successfully with potent antiretroviral therapy at the time of the first ophthalmologic examination. CD4-positive cell counts were lower and plasma HIV-RNA higher in the patient group with angiopathy. However, there were no statistically significant differences in CDC clinical stage between the two patient groups. In univariate analysis, demographic data pertaining to patients with and without angiopathy did not differ with respect to gender, age, or transmission risk (Table 1).

However, an association was found between the prevalence of retinal angiopathy, higher viral loads, and lower CD4-positive cell counts (Table 1).

In the multivariate logistic regression model, which included CD4 cell count, plasma HIV-RNA, age, gender, transmission mode, and antiretroviral therapy, a high plasma viral load was most predictive of the presence of retinal angiopathy. There was also a significant positive correlation between age and the prevalence of angiopathy, whereas the association with CD4-positive cell counts was weaker ($P = 0.051$) and did not attain statistical significance (Table 2).

Conversely, using the same model for viral retinitis, the only parameter associated with this clinical end point was a low CD4-positive cell count: the adjusted odds ratio was 0.015 (95% con-

Table 2. Association between Sociodemographic and Laboratory Variables and the Presence of Microangiopathy, Uni- and Multivariate Logistic Regression Models

	Univariate Model		Multivariate Model	
	Odds Ratio	*P Value*	*Adjusted Odds Ratio**	*P Value*
Female	1.66 (0.83–3.33)	0.16	2.34 (0.93–5.91)	0.07
Age per 10-year increase	1.19 (0.81–1.75)	0.38	1.79 (1.09–2.92)	0.02
Transmission risk				
Homosexual intercourse between males	Reference base		Reference base	
Heterosexual intercourse	1.05 (0.44–2.49)	0.91	0.59 (0.19–1.87)	0.37
Intravenous drug use	1.32 (0.56–3.08)	0.52	1.59 (0.55–4.63)	0.39
Other factors	0.52 (0.10–2.61)	0.43	0.45 (0.07–2.73)	0.38
CD4-positive cell count per 100-cell increase	0.70 (0.55–0.88)	0.004	0.76 (0.57–1.00)	0.051
HIV-1-RNA per 1-log increase	1.79 (1.31–2.44)	<0.001	1.67 (1.16–2.40)	0.005
Antiretroviral therapy	0.73 (0.36–1.48)	0.38	1.19 (0.51–2.78)	0.67

HIV = human immunodeficiency virus.
*Adjusted odds ratio for all parameters in the model.

fidence interval, 0.0007–0.30; $P = 0.006$) per 100-cell increase per microliter.

Discussion

Our data indicate that the manifestation of HIV-associated retinal microangiopathy is associated with higher HIV-RNA and thus with HIV replication.[22] Because the progression of HIV-1 infection is closely correlated with the extent of viral replication,[23] retinal microangiopathy could be a clinical marker predicting the progression of HIV infection.

Most of the patients studied had moderate to severe immunodeficiency, and more than two thirds had a history of HIV-associated opportunistic diseases. This probably reflects a certain referral bias from the HIV outpatient clinic to the ophthalmology department. Therefore, our results may not be fully generalizable to cohorts of mainly asymptomatic HIV-infected individuals.

In earlier studies, prevalence of HIV-associated retinal microangiopathy was found to be associated with CD4-positive cell counts, and thus to be a clinical marker of advanced HIV disease.[18,19,24] In univariate analysis, we confirmed this data, but when adjusting for other parameters, most importantly HIV viral load and age, this association lost statistical significance (Table 2). This points toward a pathogenetic link between viral replication and microangiopathy rather than an association of the degree of immunodeficiency with this most frequent ocular disorder in HIV infection. This may also explain why an association between retinal microangiopathy and the development of opportunistic viral ocular infections has not been demonstrated.[24]

In one study, the prevalence of cotton-wool spots was associated with HIV transmission by homosexual contact but not with age.[19] We could not demonstrate an association with transmission route of HIV, whereas the adjusted odds ratio nearly doubled for every 10-year increase of age in our analysis. An association of the prevalence of retinopathy with increasing age has also been found in the cross-sectional population-based Beaver Dan Eye Study in nondiabetic and presumably non–HIV-infected persons.[25]

Retinal microangiopathy can be found in systemic vascular disorders of degenerative, metabolic, or immunologic origin, respectively, such as in hypertension, in the elderly,[25] in diabetes mellitus,[8] in patients with systemic lupus erythematosus,[6] or in individuals treated with interferon-α.[26] We were not able to include other parameters like fibrinogen or antiphospholipid antibodies into the model because these parameters were not assessed prospectively. Of special interest is concomitant hepatitis C virus infection. Data on this infection were available for all of our patients. In the original design of the study, we did not include this factor, which was shortly afterward shown to have a certain association with retinal angiopathy.[27] In a post hoc analysis, we could not include hepatitis C in the multivariate model because this infection is very strongly correlated to a history of intravenous drug use as way of HIV transmission, the latter being in the model already. Because intravenous drug use is not significantly associated with retinal angiopathy (Table 2), we assume that we cannot show an association with hepatitis C virus infection in the studied population. However, because of the limited power of our study, we cannot definitely exclude a weak association.

Therefore, one may hypothesize that retinal microangiopathy in HIV-infected persons is a multifactorial disease superimposing HIV-related and immune-mediated vasculopathy on the background of age- or hypertension-associated microangiopathy. Our data indicate that HIV replication and the respective immune responses may be the most important factors.

Vasculopathy has been shown to be a pathologic finding in several HIV-associated conditions such as nephropathy, encephalopathy, and primary pulmonary hypertension.[28] Cerebral ischemia, either asymptomatic or leading to transient neurologic deficits, is probably more frequent in HIV-infected persons than in the general population of similar age.[29–31] Interestingly, the HIV-related ocular microangiopathic syndrome was found to be associated with impaired cognitive functioning,[32] and thus may be related to the AIDS dementia complex. Therefore, microvascular abnormalities are probably not restricted to the retina, but retinal changes may be a mirror of a systemic affection of blood vessels induced by HIV infection.

In conclusion, the presence of retinal microangiopathy is associated with a higher degree of viral replication. In patients with HIV-associated microangiopathy at presentation, unfavorable progression markers of HIV infection should be suspected and looked for. Whether successful antiretroviral combination therapy may lead to a clearance of retinal microangiopathy should be investigated in a longitudinal study.

Appendix

The members of the Swiss HIV Cohort Study are: M. Battegay (Chairman of the Scientific Board), M.-C. Bernard, E. Bernasconi, H. Bucher, Ph. Bürgisser, M. Egger, P. Erb, W. Fierz, M. Flepp (Chairman of the Clinical and Laboratory Committee), P. Francioli (President of the SHCS, Center Hospitalier Universitaire Vaudois, CH-1011 Lausanne), H. J. Furrer, M. Gorgievski, H. Günthard, P. Grob, B. Hirschel, C. Kind, Th. Klimkait, B. Ledergerber, U. Lauper, M. Opravil, F. Paccaud, G. Pantaleo, L. Perrin, J.-C. Piffaretti, M. Rickenbach (Head of Data Center), C. Rudin (Chairman of the Mother & Child Substudy), J. Schupbach, A. Telenti, P. Vernazza, Th. Wagels, and R. Weber.

References

1. Holland GN, Pepose JS, Pettit TH, et al. Acquired immune deficiency syndrome. Ocular manifestations. Ophthalmology 1983;90:859–73.
2. Jabs DA, Bartlett JG. AIDS and ophthalmology: a period of transition [editorial]. Am J Ophthalmol 1997;124:227–33.
3. Jabs DA, Green WR, Fox R, et al. Ocular manifestations of acquired immune deficiency syndrome. Ophthalmology 1989; 96:1092–9.

4. Garweg J. Opportunistic eye diseases within the scope of HIV infection. Klin Monatsbl Augenheilkd 1993;202:465–70.
5. Newsome DA, Green WR, Miller ED, et al. Microvascular aspects of acquired immune deficiency syndrome retinopathy. Am J Ophthalmol 1984;98:590–601.
6. Ushiyama O, Ushiyama K, Koarada S, et al. Retinal disease in patients with systemic lupus erythematosus. Ann Rheum Dis 2000;59:705–8.
7. Santos R, Barojas E, Alarcon-Segovia D, Ibanez G. Retinal microangiopathy in systemic lupus erythematosus. Am J Ophthalmol 1975;80:249–52.
8. Imesch PD, Bindley CD, Wallow IH. Clinicopathologic correlation of intraretinal microvascular abnormalities. Retina 1997;17:321–9.
9. Quiceno JI, Capparelli E, Sadun AA, et al. Visual dysfunction without retinitis in patients with acquired immunodeficiency syndrome. Am J Ophthalmol 1992;113:8–13.
10. Lane HC, Masur H, Edgar LC, et al. Abnormalities of B-cell activation and immunoregulation in patients with the acquired immunodeficiency syndrome. N Engl J Med 1983;309:453–8.
11. Pomerantz RJ, Kuritzkes DR, de la Monte SM, et al. Infection of the retina by human immunodeficiency virus type I. N Engl J Med 1987;317:1643–7.
12. Cantrill HL, Henry K, Jackson B, et al. Recovery of human immunodeficiency virus from ocular tissues in patients with acquired immune deficiency syndrome. Ophthalmology 1988; 95:1458–62.
13. Faber DW, Wiley CA, Lynn GB, et al. Role of HIV and CMV in the pathogenesis of retinitis and retinal vasculopathy in AIDS patients. Invest Ophthalmol Vis Sci 1992;33:2345–53.
14. Engstrom RE Jr, Holland GN, Hardy WD, Meiselman HJ. Hemorheologic abnormalities in patients with human immunodeficiency virus infection and ophthalmic microvasculopathy. Am J Ophthalmol 1990;109:153–61.
15. Pepose JS, Holland GN, Nestor MS, et al. Acquired immune deficiency syndrome. Pathogenic mechanisms of ocular disease. Ophthalmology 1985;92:472–84.
16. Glasgow BJ. Evidence for breaches of the retinal vasculature in acquired immune deficiency syndrome angiopathy. A fluorescent microsphere study. Ophthalmology 1997;104:753–60.
17. Kiener M, Garweg JG, Furrer H, Malinverni R. HIV-assozierte retinale Mikroangiopathie und die Entwicklung AIDS-definierender Infektionen. Die Berner Angiopathie-Studie. Klin Monatsbl Augenheilkd 1998;212:345–9.
18. Freeman WR, Chen A, Henderly DE, et al. Prevalence and significance of acquired immunodeficiency syndrome-related retinal microvasculopathy. Am J Ophthalmol 1989;107:229–35.
19. Spaide RF, Gaissinger A, Podhorzer JR. Risk factors for cotton-wool spots and for cytomegalovirus retinitis in patients with human immunodeficiency virus infection. Ophthalmology 1995;102:1860–4.
20. Sudre P, Rickenbach M, Taffé P, et al. Clinical epidemiology and research on HIV infection in Switzerland: the Swiss HIV Cohort Study 1988–2000. Schweiz Med Wochenschr 2000; 130:1493–500.
21. Garweg J, Fenner T, Bohnke M, Schmitz H. An improved technique for the diagnosis of viral retinitis from samples of aqueous humor and vitreous. Graefes Arch Clin Exp Ophthalmol 1993;231:508–13.
22. Ho DD, Neumann AU, Perelson AS, et al. Rapid turnover of plasma virions and CD4 lymphocytes in HIV-1 infection. Nature 1995;373:123–6.
23. Mellors JW, Munoz A, Giorgi JV, et al. Plasma viral load and CD4+ lymphocytes as prognostic markers of HIV-1 infection. Ann Intern Med 1997;126:946–54.
24. Kuppermann BD, Petty JG, Richman DD, et al. Correlation between CD4+ counts and prevalence of cytomegalovirus retinitis and human immunodeficiency virus-related noninfectious retinal vasculopathy in patients with acquired immunodeficiency syndrome. Am J Ophthalmol 1993;115:575–82.
25. Klein R, Klein BEK, Moss SE, Wang Q. Hypertension and retinopathy, arteriolar narrowing, and arteriovenous nicking in a population. Arch Ophthalmol 1994;112:92–8.
26. Hejny C, Sternberg P, Lawson DH, et al. Retinopathy associated with high-dose interferon alfa-2b therapy. Am J Ophthalmol 2001;131:782–7.
27. Thierfelder S, Linnert D, Grehn F. Increased prevalence of HIV-related retinal microangiopathy syndrome in patients with hepatitis C [letter]. Arch Ophthalmol 1996;114:899.
28. Terada LS, Gu Y, Flores SC. AIDS vasculopathy. Am J Med Sci 2000;320:379–87.
29. Qureshi AI, Janssen RS, Karon JM, et al. Human immunodeficiency virus infection and stroke in young patients. Arch Neurol 1997;54:1150–3.
30. Pinto AN. AIDS and cerebrovascular disease. Stroke 1996;27: 538–43.
31. Brew BJ, Miller J. Human immunodeficiency virus type 1-related transient neurological deficits. Am J Med 1996;101:257–61.
32. Geier SA, Perro C, Klauss V, et al. HIV-related ocular microangiopathic syndrome and cognitive functioning. J Acquir Immune Defic Syndr 1993;6:252–8.