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Authors

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Nguyen, Minh Ly
Potter, Michael
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Publication Date

2019-02-01

DOI

10.1016/j.jim.2018.11.008

Peer reviewed

Journal of Immunological Methods xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Journal of Immunological Methods

journal homepage: www.elsevier.com/locate/jim

Research paper

Comparison of bead array and glass nanoreactor multi-analyte platforms for the evaluation of CNS and peripheral inflammatory markers during HIV infection

Albert M. Anderson[a,*], Minh Ly Nguyen[a], Michael Potter[b,c], Debra Rosario[b,c], Katarzyna Kempinska[d], Ronald J. Ellis[e], Mitchell Diccianni[d], Scott L. Letendre[b,c]

[a] Department of Medicine, Division of Infectious Diseases, Emory University School of Medicine, Atlanta, GA, United States
[b] Department of Medicine, University of California at San Diego School of Medicine, La Jolla, CA, United States
[c] Department of Psychiatry, University of California at San Diego School of Medicine, La Jolla, CA, United States
[d] Department of Pediatrics, University of California at San Diego School of Medicine, La Jolla, CA, United States
[e] Department of Neurosciences, University of California at San Diego School of Medicine, La Jolla, CA, United States

ARTICLE INFO

Keywords:
HIV
Cerebrospinal fluid
Tumor necrosis factor-alpha
Monocyte chemotactic protein-1/chemokine CCL2
Fractalkine/chemokine CX3CL1

ABSTRACT

While human immunodeficiency virus (HIV) infection has become a treatable disease with the development of combination antiretroviral therapy (cART), chronic inflammation that affects the central nervous system and other organs is still common. Reliable methods are needed to study HIV-associated inflammatory biomarkers. In this study involving both plasma and cerebrospinal fluid (CSF), we compared multiplex bead array (MBA) to a relatively new technology based on microfluidics and glass nanoreactor (GNR) technology for the measurement of three commonly studied markers from HIV-infected individuals. We found that results correlated between the two platforms for MCP-1 in both fluids as well as for plasma TNFα (all $p < .005$). However, results between the two platforms did not correlate for CSF TNFα or fractalkine from plasma or CSF. A statistically significant decrease in CSF TNFα over time ($p < .0001$) was only detectable with the MBA platform, and TNFα on the MBA was the only CSF biomarker to correlate with CSF HIV RNA (rho = 0.71, $p < .0001$). Meanwhile, the GNR platform was superior in terms of intra-assay fractalkine (FKN) variability and the detection of a significant FKN decrease over time. Additionally, the only significant correlation between blood biomarkers and plasma HIV RNA was with FKN on the GNR platform (rho = 0.38, $p = .015$). Given the variability in results between platforms, more research is needed on methods to quantitate HIV-associated inflammation.

1. Introduction

While combination antiretroviral therapy (cART) has transformed HIV infection into a treatable disease, the infection is linked to an increase in systemic inflammation despite optimal virologic suppression during cART (Neuhaus et al., 2010). This persistent inflammatory state is associated with multiple unfavorable clinical outcomes, including increased mortality (Duprez et al., 2012). Numerous central nervous system (CNS) findings during HIV have also been linked to inflammation. Specifically, HIV-associated neurocognitive disorder (HAND), which continues to be highly prevalent in the cART era (Heaton et al., 2010), appears to have inflammatory underpinnings (Burdo et al., 2013; Saylor et al., 2016). Additionally, inflammation during HIV has been associated with neuroimaging findings of decreased neuronal integrity, glial proliferation, and membrane turnover (Anderson et al., 2015a; Anderson et al., 2015b). However, multiple different inflammatory pathways appear to be dysregulated during HIV (Deeks et al., 2013), meaning that a more comprehensive understanding of inflammation is needed to most effectively address the long term effects of the infection. This includes more research on how HIV-associated inflammation changes over time, which has become a surrogate outcome in early phase studies of anti-inflammatory agents to treat individuals with chronic HIV (Probasco et al., 2008).

Methods that are highly automated yet sensitive may facilitate the study of HIV-associated inflammatory markers by allowing for easier scalability and the minimization of operator error. Currently available methods include (but are not limited to) enzyme linked immunosorbent assay (ELISA), chemiluminescence, and multiplex bead array (MBA). One version of MBA relies on multiple different colors of beads, with each unique color bead being coupled to a single analyte-specific

* Corresponding author at: Emory University School of Medicine, 341 Ponce de Leon Avenue, Atlanta, GA 30308, United States.
E-mail address: aande2@emory.edu (A.M. Anderson).

https://doi.org/10.1016/j.jim.2018.11.008
Received 10 September 2018; Received in revised form 19 November 2018; Accepted 19 November 2018

antibody (Luminex xMAP technology, Luminex corp, Austin, Texas). Fluorescence levels are then quantitated after laser interrogation. A newer method for the quantitation of inflammatory markers is based on microfluidics and glass nanoreactor (GNR) technology (Simple Plex, Protein Simple corp, San Jose, California). Briefly, samples are loaded into a cartridge with multiple microfluidic circuits, with each circuit having lanes with a specific detection antibody for each analyte (Aldo et al., 2016). Each lane has three GNR's through which the sample travels, resulting in a triplicate reading for each sample. Within the GNR, analytes are immobilized with capture antibodies and fluorescence is then measured after administration of streptavidin-associated dye. Specific standard curves are generated for each cartridge lot by the company prior to shipment.

For the current study, we compared MBA and GNR platforms for the quantitation of inflammatory markers from the paired plasma and cerebrospinal fluid (CSF) samples of HIV-infected participants before and after initiation of cART. Three markers that have been associated with HAND and are common between the two platforms were selected: monocyte chemotactic protein 1 (MCP-1, also known as CCL-2), tumor necrosis factor alpha (TNFα), and fractalkine (FKN, also known as CX3CL1). MCP-1 is a chemokine that attracts/activates monocytes and has been associated with both decreased neuropsychological performance and markers of neuronal injury during HIV (Woods et al., 2006; Anderson et al., 2015b). TNFα is a potent pro-inflammatory cytokine that remains elevated in the blood throughout the course of HIV infection (Wada et al., 2015). This cytokine has neurotoxic properties and has also been associated with HAND (Sevigny et al., 2004). FKN is a chemokine which attracts multiple immune cells, including microglia. This chemokine is upregulated in the brain and CSF of individuals with HIV-associated dementia (Cotter et al., 2002). In vivo models have shown that FKN receptor deficiency dysregulates microglial responses, resulting in neurotoxicity (Cardona et al., 2006). Therefore, it has been postulated that FKN may have an important role in neuroprotection.

2. Materials and methods

2.1. Study participants

Participants were enrolled between 2013 and 2017 at the Emory University Center for AIDS Research (CFAR) clinical core site in Atlanta as part of ongoing studies on HIV and neurocognition, in which participants off cART (either cART naïve or off cART for at least six months) were enrolled and then had a second visit 24–48 weeks after the initiation of therapy. Individuals with chronic HIV between 18 and 59 years of age were eligible for participation. Individuals were excluded from the study for any of the following: 1) history of any neurologic disease known to affect memory (including stroke, malignancy involving the brain, traumatic brain injury, and AIDS-related opportunistic infection of the central nervous system); 2) current ongoing substance use (marijuana use in the last 7 days OR cocaine, heroin, methamphetamine, or other non-marijuana illicit drug use in the last 30 days); 3) heavy alcohol consumption in the last 30 days (defined as > 7 drinks per week for women and > 14 drinks per week for men); or 4) serious mental illness including schizophrenia and bipolar disorder (depression was not excluded if participants were well controlled on treatment). HIV+ participants with a history of treated syphilis and a persistently positive rapid plasma reagin (RPR) titer of 1:8 or less were eligible for the study if there was a decrease in RPR of at least fourfold at six months after treatment and there were no neurological symptoms at initial syphilis presentation. Institutional Review Board-approved informed consent was obtained from all participants.

2.2. Sample processing and laboratory protocol

Blood and CSF samples came from the same visit on the same chronological date. Samples were processed with a standard protocol at the Emory Infectious Diseases Clinical Research Laboratory. Specifically, blood is collected in Becton Dickinson ACD citrated tubes. Tubes are then centrifuged at 2400 rotations per minute (RPM) for 10 min. Plasma supernatant is then removed and re-spun at 2400 RPM for an additional 10 min. The resulting sample is then aliquoted into cryovials and then stored in a −80 °C freezer. Therefore, plasma samples are not specifically platelet free. CSF is collected in sterile polypropylene tubes and then centrifuged at 300 relative centrifugal force for 15 min. The resulting sample is then aliquoted into cryovials and also stored in a −80 °C freezer.

All assays were performed during the same seven day period for both platforms by the same laboratory personnel. All MBA kits were from the same lot, and all GNR kits were from the same lot. Samples were run in duplicate with the MBA platform, while as mentioned previously, the GNR platform automatically creates a triplicate for each sample that is loaded. While GNR cartridges are only available through Protein Simple, MBA kits and plates were purchased through Millipore Sigma (Burlington, Massachusetts). Assays for both platforms were performed as per company protocol instructions with no deviations. For the MBA platform, the standards provided by the company are reconstituted with 250 µl of deionized water to give a 10,000 picogram/ml (pg/ml) concentration. Five subsequent serial dilutions are then made which represent 20% of each preceding concentration. After application of wash buffer to each well the participant samples, 25 µl of participant sample as well as standard and control are used for each well and combined with the pre-mixed beads. There is no dilution of the samples with the MBA platform. After processing, individual plates are run on the Luminex Flexmap 3D system. There are two control samples provided with the MBA kits. The between-plate CV% for control #1 (which represented lower values) were 4.56 for MCP-1, 20.3 for TNFα, and 85.3 for FKN. The between-plate CV% for control #2 (which represented higher values) were 3.3 for MCP-1, 2.7 for TNFα, and 36.3 for FKN. Please note that the Flexmap 3D system does not adjust results of plate samples on the basis of the control results.

For the GNR platform, the company protocol was again used with no deviations. Briefly, the barcode for the cartridge provided by the company is scanned into the device (which is named “Ella”). One milliliter of wash buffer is then added to each well. Like the MBA platform, 25 µl of participant sample is needed for each well. In contrast to the MBA platform, a 1:2 dilution of participant sample is used for each well. The standards come pre-loaded in the cartridge by the company. Unlike the MBA system, there are no controls provided for the GNR platform unless specifically requested. The cartridge is then placed into the Ella device and processed automatically with no interruptions.

Lowest limits of detection as provided by the company for the MBA assays were: 1.9 picograms (pg)/ milliliter (ml) for MCP-1, 0.7 pg/ml for TNFα, and 22.7 pg/ml for FKN. Lowest limits of detection as provided by the company for the GNR assays were: 0.35 pg/ml for MCP-1, 0.278 pg/ml for TNFα, and 18.2 pg/ml for FKN. The coefficient of variation (CV) % is derived automatically by both individual platforms with no manual calculations ((Standard deviation/mean)x100).

2.3. Statistical analyses

SAS JMP software version 13 was used for analyses and SAS IML studio 13 was used for graphics. Normality of variable distribution was assessed with the Shapiro-Wilk test. Given the skewed distribution of most variables, comparisons between continuous variables were performed with the Wilcoxon rank sum test. For same-visit and longitudinal paired variable comparison, the Wilcoxon signed rank test was used. For categorical variables, Fisher's exact test was used. For correlations, Spearman's rho test was used. Alpha level for significance was set at < 0.05 and was two tailed.

Table 1
Demographic and Disease characteristics of participants.

Variable (N = 41)	Median (IQR) or Number (%)
Age in years	40 (33–47)
Male Sex	33 (80.5%)
Race	
African American	36 (87.8%)
White	5 (12.2%)
Blood Tests	
CD4+ Current (per μl)	94 (30 − 302)
Creatinine (mg/dl)	0.9 (0.8–1.1)
HIV RNA (log10 copies/ml)	4.61 (4.0–5.27)
CSF Tests	
WBC (per μl)	1 (0–6)
RBC (per μl)	0 (0–1.5)
HIV RNA (log10 copies/ml)	3.1 (2.1–3.77)

IQR = interquartile range; CD = cluster of differentiation, μl = microliter; mg = milligrams; dl = deciliters; ml = milliliters; HIV = human immunodeficiency virus; RNA = ribonucleic acid; CSF = cerebrospinal fluid; WBC = white blood cell count; RBC = red blood cell count.

Table 2
Visit 1 biomarker comparisons.

Analyte (n = 41)	Fluid	Platform	CV in % Median (IQR)	Correlation between platforms (p value)
MCP-1	Plasma	MBA	2.8 (1.4–4.8)	0.69 (< 0.0001)
		GNR	3.2 (1.7–3.6)	
MCP-1	CSF	MBA	2.4 (0.9–4.1)	0.74 (< 0.0001)
		GNR	4.5 (2.8–6.3)*	
TNFα	Plasma	MBA	5.5 (2.3–8.9)	0.5 (0.001)
		GNR	2.2 (1.3–2.6)*	
TNFα	CSF	MBA	4.6 (3.0–10.2)	−0.04 (0.82)
		GNR	2.3 (1.0–11.2)	
Fractalkine	Plasma	MBA	8.3 (4.1–15.2)	0.1 (0.6)
		GNR	1.8 (1.2–2.3)*	
Fractalkine	CSF	MBA	17.4 (8.5–29.9)	– –
		GNR	5.5 (3.3–9.1)*	

CV = coefficient of variation; IQR = interquartile range; MBA = multiplex bead array platform; GNR = glass nanoreactor platform; CSF = cerebrospinal fluid; * denotes p < .005 for CV difference between platforms; Spearman's rho used for correlation. – denotes that the majority of CSF fractalkine values were undetectable with MBA platform.

3. Results

There were 41 HIV-infected individuals at baseline, with 29 returning for repeat testing 24–48 weeks after starting cART. Table 1 shows baseline demographic and disease characteristics. The median age was 40 years and the majority of participants (75.6%) were African-American men. Comorbidities were minimal (three participants had hypertension, two participants had chronic hepatitis B virus (HBV) infection, one participant had chronic hepatitis C virus (HCV) infection, and one participant had diabetes). Both assays yielded consistently detectable concentrations at both visits for MCP-1 in both fluids and for TNFα from plasma. CSF MCP-1 concentrations were consistently higher at both visits with the MBA assay (both $p < .0001$), and plasma TNFα concentrations were also consistently higher at both visits with the MBA assay (both $p < .0001$). With the MBA platform, CSF TNFα concentrations were all detectable (100%) at visit one and detectable for all but one (97%) at visit two. With the GNR platform, 34 of 41 (82.9%) samples were detectable for CSF TNFα at visit one and 23 of 29 (79.3%) samples were detectable at visit two. Therefore, CSF TNFα was more likely to be detectable with the MBA platform at the first visit ($p = .01$) with a trend towards higher detectability at the second visit ($p = .1$). All but one sample yielded detectable FKN concentrations for both fluids at both visits for the GNR platform. For the MBA platform, plasma FKN was detectable in 37 of 41 visit one samples (90.2%) and 28 of 29 visit two samples (96.6%), meaning that plasma FKN detectability was not significantly different between platforms. However, CSF FKN concentrations on the MBA assay were below the limit of detection in 36 of 41 visit one samples (87.8%) and 24 of 29 visit two samples (82.8%), meaning that CSF FKN detectability was significantly higher for the GNR assay at both visits ($p < .0001$).

In Table 2, biomarker coefficient of variation (CV) results and between-platform correlations are shown for visit 1. For MCP-1, concentrations correlated significantly between platforms for both fluids (see Fig. 1 for plasma correlation with 80% density ellipse, with units being picograms/ml for both axes), and the coefficient of variation (CV) was significantly lower with the MBA platform for the measurement of CSF MCP-1. For TNFα, only plasma concentrations correlated significantly between the two platforms, and the CV was significantly lower with the GNR platform with this fluid. Neither plasma nor CSF FKN concentrations correlated significantly between the two platforms, and CV was significantly lower with the GNR platform for the measurement of both plasma and CSF FKN. Neither platform yielded a significant correlation between plasma MCP-1 and log10 plasma HIV RNA or a significant correlation between CSF MCP-1 and log10 CSF HIV RNA. The only significant TNF-HIV correlation was between CSF TNFα and log10 CSF HIV RNA using the MBA platform ($rho = 0.71$, $p < .0001$, see Fig. 2). The only significant FKN-HIV correlation was between plasma FKN and log10 plasma HIV RNA using the GNR platform ($rho = 0.38$, $p = .015$, see Fig. 3).

At visit 2 (Table 3), 22 of 29 participants (76%) had achieved both plasma and CSF HIV RNA < 400 copies/ml. Just as in visit 1, both plasma and CSF MCP-1 concentrations as well as plasma TNFα concentrations correlated significantly between the two platforms (all $p < .005$). Again, however, CSF TNFα concentrations as well as both plasma and CSF FKN concentrations did not correlate significantly between the two platforms. FKN CV was again significantly lower with the GNR platform for both plasma and CSF. Between visits, both platforms detected statistically significant decreases in plasma and CSF MCP-1 as well as plasma TNFα after initiation of cART (see Table 4). While the MBA platform detected a statistically significant decrease in CSF TNFα, the GNR platform did not, even when excluding participants who had an undetectable concentration at the first visit. In contrast, only the GNR platform detected a statistically significant decrease in plasma FKN.

4. Discussion

Until a cure is eventually found, HIV will very likely continue to be a pro-inflammatory disease that is associated with adverse outcomes. These include adverse CNS effects such as neuronal damage and HIV-associated neurocognitive disorder. Therefore, high sensitivity methods that are scalable and precise will be needed to measure inflammatory biomarkers, including from the CNS. In this study, we evaluated a relatively new platform based on glass nanoreactor (GNR) technology in relationship to the established multiplex bead array (MBA) as a means to quantitate HIV-associated biomarkers from both CSF and plasma. The GNR platform is a novel technology based on microfluidics. To our knowledge, this is the first study evaluating the GNR platform for the quantitation of CSF biomarkers during HIV infection.

We found that MCP-1 concentrations from both plasma and CSF consistently correlated between the two platforms, as did TNFα concentrations from plasma. However, CSF TNFα concentrations (which only correlated with CSF HIV RNA level using the MBA platform and for which significant change could only be detected with the MBA platform) did not correlate between platforms. While TNFα concentrations from blood are known to decrease after initiation of cART (McComsey et al., 2014), TNFα remains elevated despite long term HIV suppression (Wada et al., 2015). TNFα has long been recognized as a neurotoxic cytokine with damaging effects on both neurons and oligodendrocytes

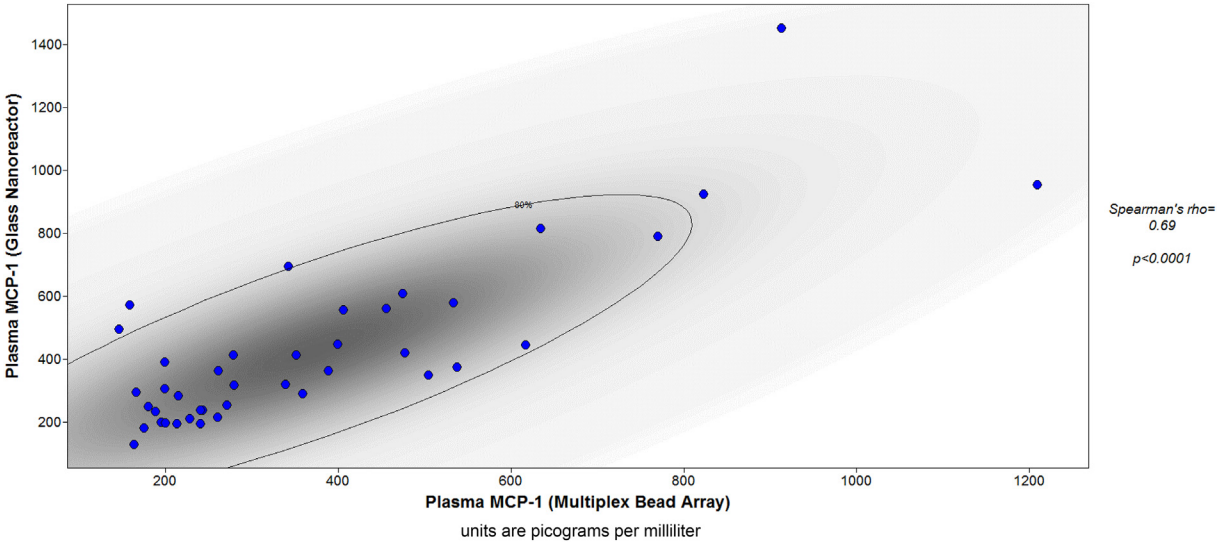

Fig. 1. Correlation graph with 80% density ellipse between plasma monocyte chemoattractant protein-1 (MCP-1) concentrations obtained by multiplex bead array assay (Luminex platform, on x-axis) and plasma MCP-1 concentrations obtained by glass nanoreactor assay (Protein Simple platform, on y-axis). Units for both axes are picograms/milliliter. Results are from HIV-infected participants off antiretroviral treatment.

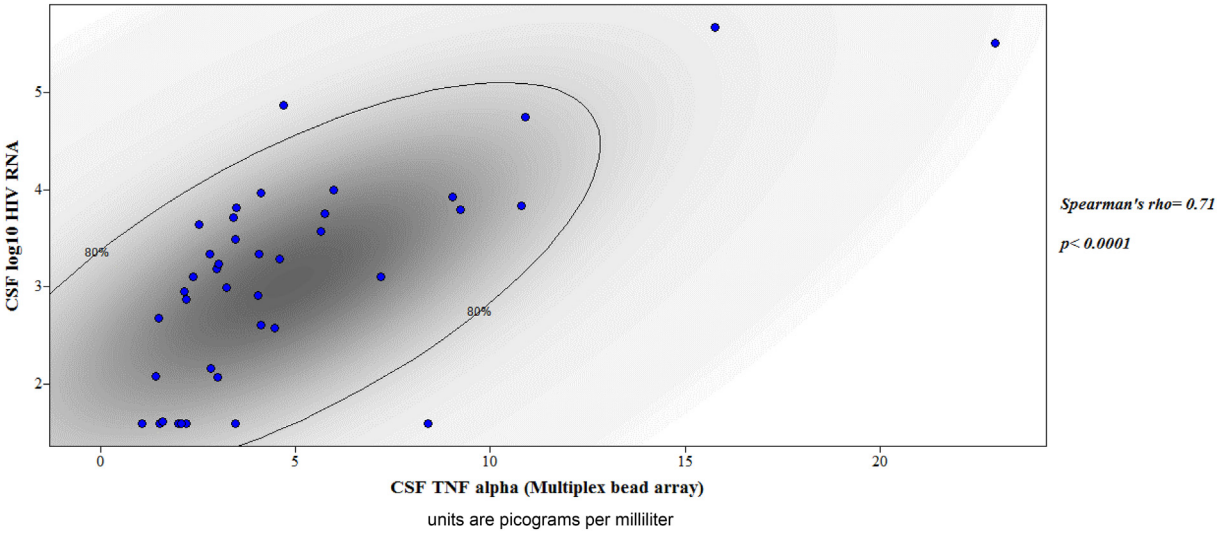

Fig. 2. Correlation graph with 80% density ellipse between cerebrospinal fluid (CSF) tumor necrosis factor alpha concentrations in picograms/milliliter obtained by multiplex bead array assay (Luminex platform, on x-axis) and CSF HIV RNA concentrations (copies/milliliter, log10 transformed) obtained by polymerase chain reaction (Abbott laboratories m2000 Real Time HIV-1 assay system, on y-axis). Results are from HIV-infected participants off antiretroviral treatment.

(Selmaj and Raine, 1988). It is elevated in the CSF of individuals with diseases such as sub-arachnoid hemorrhage and progressive multiple sclerosis (Rossi et al., 2014; Wu et al., 2016). In early pre-cART research, CSF TNFα concentrations correlated significantly with CSF HIV RNA (Lafeuillade et al., 1996). In our study, this finding was only replicated with the MBA platform. Based on our findings, the MBA platform may be preferable for measuring this cytokine from CSF.

In contrast, we found that the GNR platform was significantly more sensitive than the MBA platform for the measurement of FKN, with higher intra-assay precision and capability to detect significant change over time. Plasma FKN only correlated with plasma HIV using the GNR platform. FKN is a potent chemokine that attracts and activates leukocytes such as brain macrophages and microglia, and has been shown to be expressed at higher levels in the CNS compared to the periphery (Bazan et al., 1997; Cotter et al., 2002). In addition to an association with HIV infection, CSF FKN concentrations have been found to be elevated in other diseases such as neuropsychiatric systemic lupus erythematosus (SLE) (Yajima et al., 2005). While some studies show that FKN has neuroprotective properties, others point to neurotoxic properties based on upregulation of inflammation (Lauro et al., 2015). Therefore, further study is needed to better understand the effects of this chemokine during HIV and other diseases. Based on our findings, the GNR platform may be preferable for measuring FKN from both plasma and CSF.

We acknowledge the limitations of this small study. There are many other markers that have been found to be altered during HIV, including C-reactive protein (CRP), IL-6, soluble CD14 and soluble CD163 (Hunt et al., 2016). Examination of these markers would have strengthened the study, but these examinations would have been beyond the scope of this research project, which had specific aims and funding. Future studies that compare biomarker platforms should consider including these additional markers. While three markers correlated significantly between the two platforms, the values were far from being exactly the same, with the highest Spearman's correlation coefficient being 0.74. All of the samples were run over a single seven day period with the same laboratory personnel. The kits that were used for each platform

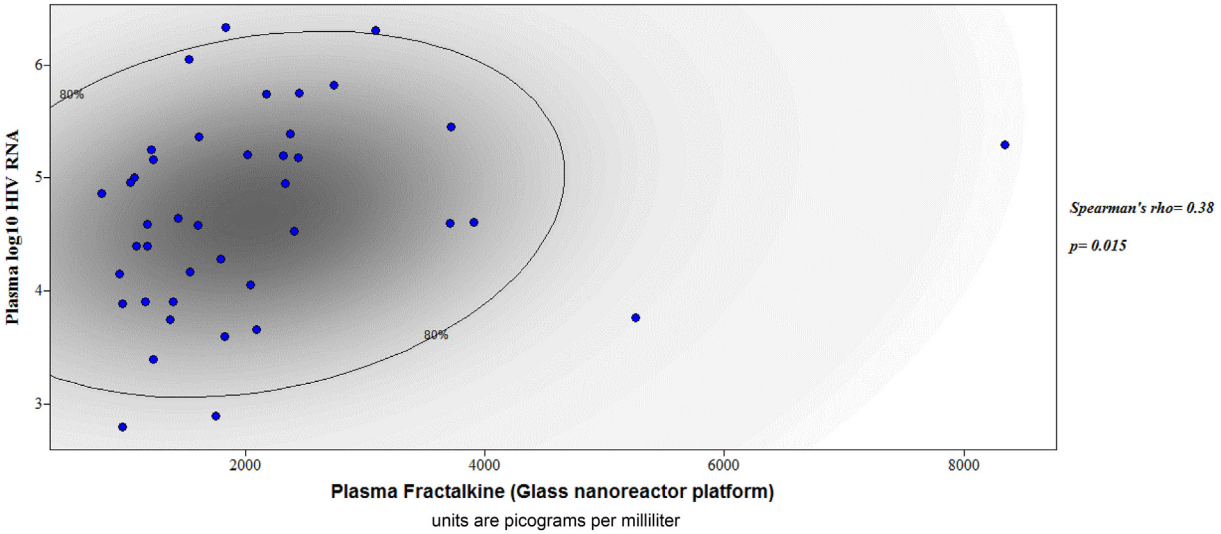

Fig. 3. Correlation graph with 80% density ellipse between plasma fractalkine concentrations (in picograms/milliliter) obtained by glass nanoreactor assay (Protein Simple platform, on x-axis) and plasma HIV RNA concentrations (copies/milliliter, log10 transformed) obtained by polymerase chain reaction (Abbott laboratories m2000 Real Time HIV-1 assay system, on y-axis). Results are from HIV-infected participants off antiretroviral treatment.

Table 3
Visit 2 biomarker comparisons.

Analyte (n = 29)	Fluid	Platform	CV in % Median (IQR)	Correlation between platforms (p value)
MCP-1	Plasma	MBA	3.6 (1.4–6.8)	0.74 (< 0.0001)
		GNR	2.5 (1.0–3.2)	
MCP-1	CSF	MBA	2.1 (1.3–3.3)	0.64 (0.0002)
		GNR	3.3 (2.1–4.8)	
TNFα	Plasma	MBA	3.5 (1.2–8.2)	0.53 (0.004)
		GNR	2.3 (0.9–4.3)	
TNFα	CSF	MBA	8.5 (5.4–14.9)	−0.25 (0.26)
		GNR	1.1 (0.3–8.3)	
Fractalkine	Plasma	MBA	15.4 (7.2–23.2)	−0.01 (0.95)
		GNR	1.8 (1.1–2.7)*	
Fractalkine	CSF	MBA	24.4 (10.2–36.1)	– –
		GNR	6.5 (3.8–10.3)*	

CV = coefficient of variation; IQR = interquartile range; MBA = multiplex bead array platform; GNR = glass nanoreactor platform; CSF = cerebrospinal fluid; * denotes p < .005 for CV difference between platforms; Spearman's rho used for correlation. – denotes that the majority of CSF fractalkine values were undetectable with MBA platform.

Table 4
Biomarker change between visits.

Analyte (n = 29)	Fluid	Platform	Median change between visits (IQR)	P value
MCP-1	Plasma	MBA	−56.0 (−190.8–+21.5)	0.004
		GNR	−93.8 (−219.2 to −31.7)	0.0001
MCP-1	CSF	MBA	−471.0 (−1026.0 to −120.5)	< 0.0001
		GNR	−48.6 (−142.7–+8.3)	0.009
TNFα	Plasma	MBA	−6.4 (−13.5–+0.6)	0.001
		GNR	−5.5 (−8.0 to −2.0)	< 0.0001
TNFα	CSF	MBA	−1.8 (−3.0 to −0.5)	< 0.0001
		GNR	0.0 (−0.7–+0.9)	0.95
Fractalkine	Plasma	MBA	+9.0 (−35.7–+118.6)	0.27
		GNR	−275.4 (−924.8 to −88.8)	0.001
Fractalkine	CSF	MBA	– –	–
		GNR	+6.5 (−38.4–+63.8)	0.48

MBA = multiplex bead array platform; GNR = glass nanoreactor platform; CSF = cerebrospinal fluid; – - denotes that change could not be calculated due to majority of values being undetectable.

were from the same lot. The coefficient of variation (CV) % was derived automatically by both platforms with no manual calculations ((Standard deviation/mean) x 100). However, we did not examine inter-assay precision as would be determined, for example, by measurement of the same samples on different days. Therefore our results do not take into account differences based on the day the assay was performed. Also, we did not examine accuracy as would be determined by quantitating markers from samples with previously determined concentrations.

The inclusion of a third, more traditional method such as ELISA would have strengthened the study by providing a standard to which the two newer platforms could be compared. However, this would have been beyond the scope of the project, which had specific grant funded aims. Pipetting and other factors that influence the preparation of plates may have influenced CV% difference between the two platforms. Specifically, the MBA platform requires two separate wells for the duplicate values that are produced. In contrast, the GNR platform does not require separate wells for the triplicate values that are produced. Rather, these triplicate values are produced automatically as the sample travels through the three glass nanoreactors that are associated with each analyte. Therefore, it is possible that having to create two separate wells may influence the CV% that are produced with the MBA platform. There was a particularly strong difference in CV% values for FKN between the two platforms, and this difference in replicate production between platforms may have played a role.

The sample size of the study was relatively limited, meaning that small differences between the platforms may have been missed. Due to small sample size, we did not examine biomarker associations with neuropsychological performance. The persistence of neurocognitive impairment continues to be problematic in the cART era, and a better understanding of how inflammation influences impairment is needed. We and other groups are working to address this question in larger biomarker studies with more power to detect significant effects. Other than differences in performance, a number of other factors (including time and cost) play a role when investigators decide how to measure soluble biomarkers. As mentioned, the GNR platform cartridges come with pre-established factory calibrated standard curves. For this and other reasons related to the setup of the assay, the GNR plates are more automated and require < 50% of the time to run than the MBA plates in our experience. These characteristics could reduce institution-to-institution, lab-to-lab and user-to-user variability. However, the GNR kits were > 50% more expensive than the MBA kits in our experience. Cost is often a major consideration, particularly when performing large

numbers of samples. Lastly, multiple other companies offer MBA kits, and the scope of this project was not broad enough to compare kits from multiple different vendors on the MBA platform.

5. Conclusions

Fractalkine results did not correlate between MBA and GNR platforms for plasma or CSF, nor did TNFα results from CSF. The GNR platform performance appeared to be superior for the quantitation of FKN, while the MBA platform performance appeared to be superior for CSF TNFα quantitation. Overall, more research is needed on the similarities and differences of platforms for the measurement of immunologic markers in HIV and other disease states.

Acknowledgements

This work was funded by the following National Institutes of Health (NIH) grants: K23MH095679, K24MH097673, and P30AI050409 (Emory Center for AIDS Research).

Declarations of interest

None.

References

Aldo, P., Marusov, G., Svancara, D., David, J., Mor, G., 2016. Simple Plex(™) : a novel multi-analyte, automated microfluidic immunoassay platform for the detection of human and mouse cytokines and chemokines. Am. J. Reprod. Immunol. 75, 678–693.

Anderson, A.M., Harezlak, J., Bharti, A., Mi, D., Taylor, M.J., Daar, E.S., Schifitto, G., Zhong, J., Alger, J.R., Brown, M.S., Singer, E.J., Campbell, T.B., McMahon, D.D., Buchthal, S., Cohen, R., Yiannoutsos, C., Letendre, S.L., Navia, B.A., Consortium, H.I.V.N, 2015a. Plasma and cerebrospinal fluid biomarkers predict cerebral injury in HIV-infected individuals on stable combination antiretroviral therapy. J. Acquir. Immune Defic. Syndr. 69, 29–35.

Anderson, A.M., Fennema-Notestine, C., Umlauf, A., Taylor, M.J., Clifford, D.B., Marra, C.M., Collier, A.C., Gelman, B.B., McArthur, J.C., McCutchan, J.A., Simpson, D.M., Morgello, S., Grant, I., Letendre, S.L., Group, C, 2015b. CSF biomarkers of monocyte activation and chemotaxis correlate with magnetic resonance spectroscopy metabolites during chronic HIV disease. J. Neurovirol. 21, 559–567.

Bazan, J.F., Bacon, K.B., Hardiman, G., Wang, W., Soo, K., Rossi, D., Greaves, D.R., Zlotnik, A., Schall, T.J., 1997. A new class of membrane-bound chemokine with a CX3C motif. Nature 385, 640–644.

Burdo, T.H., Weiffenbach, A., Woods, S.P., Letendre, S., Ellis, R.J., Williams, K.C., 2013. Elevated sCD163 in plasma but not cerebrospinal fluid is a marker of neurocognitive impairment in HIV infection. AIDS 27, 1387–1395.

Cardona, A.E., Pioro, E.P., Sasse, M.E., Kostenko, V., Cardona, S.M., Dijkstra, I.M., Huang, D., Kidd, G., Dombrowski, S., Dutta, R., Lee, J.C., Cook, D.N., Jung, S., Lira, S.A., Littman, D.R., Ransohoff, R.M., 2006. Control of microglial neurotoxicity by the fractalkine receptor. Nat. Neurosci. 9, 917–924.

Cotter, R., Williams, C., Ryan, L., Erichsen, D., Lopez, A., Peng, H., Zheng, J., 2002. Fractalkine (CX3CL1) and brain inflammation: Implications for HIV-1-associated dementia. J. Neuro-Oncol. 8, 585–598.

Deeks, S.G., Tracy, R., Douek, D.C., 2013. Systemic effects of inflammation on health during chronic HIV infection. Immunity 39, 633–645.

Duprez, D.A., Neuhaus, J., Kuller, L.H., Tracy, R., Belloso, W., De Wit, S., Drummond, F., Lane, H.C., Ledergerber, B., Lundgren, J., Nixon, D., Paton, N.I., Prineas, R.J., Neaton, J.D., Group, I.S.S, 2012. Inflammation, coagulation and cardiovascular disease in HIV-infected individuals. PLoS One 7, e44454.

Heaton, R.K., Clifford, D.B., Franklin Jr., D.R., Woods, S.P., Ake, C., Vaida, F., Ellis, R.J., Letendre, S.L., Marcotte, T.D., Atkinson, J.H., Rivera-Mindt, M., Vigil, O.R., Taylor, M.J., Collier, A.C., Marra, C.M., Gelman, B.B., McArthur, J.C., Morgello, S., Simpson, D.M., McCutchan, J.A., Abramson, I., Gamst, A., Fennema-Notestine, C., Jernigan, T.L., Wong, J., Grant, I., Group, C, 2010. HIV-associated neurocognitive disorders persist in the era of potent antiretroviral therapy: CHARTER Study. Neurology 75, 2087–2096.

Hunt, P.W., Lee, S.A., Siedner, M.J., 2016. immunologic biomarkers, morbidity, and mortality in treated HIV infection. J. Infect. Dis. 214 (Suppl. 2), S44–S50.

Lafeuillade, A., Poggi, C., Pellegrino, P., Corti, K., Profizi, N., Sayada, C., 1996. HIV-1 replication in the plasma and cerebrospinal fluid. Infection 24, 367–371.

Lauro, C., Catalano, M., Trettel, F., Limatola, C., 2015. Fractalkine in the nervous system: neuroprotective or neurotoxic molecule? Ann. N. Y. Acad. Sci. 1351, 141–148.

McComsey, G.A., Kitch, D., Sax, P.E., Tierney, C., Jahed, N.C., Melbourne, K., Ha, B., Brown, T.T., Bloom, A., Fedarko, N., Daar, E.S., 2014. Associations of inflammatory markers with AIDS and non-AIDS clinical events after initiation of antiretroviral therapy: AIDS clinical trials group A5224s, a substudy of ACTG A5202. J. Acquir. Immune Defic. Syndr. 65, 167–174.

Neuhaus, J., Jacobs Jr., D.R., Baker, J.V., Calmy, A., Duprez, D., La Rosa, A., Kuller, L.H., Pett, S.L., Ristola, M., Ross, M.J., Shlipak, M.G., Tracy, R., Neaton, J.D., 2010. Markers of inflammation, coagulation, and renal function are elevated in adults with HIV infection. J. Infect. Dis. 201, 1788–1795.

Probasco, J.C., Spudich, S.S., Critchfield, J., Lee, E., Lollo, N., Deeks, S.G., Price, R.W., 2008. Failure of atorvastatin to modulate CSF HIV-1 infection: results of a pilot study. Neurology 71, 521–524.

Rossi, S., Motta, C., Studer, V., Barbieri, F., Buttari, F., Bergami, A., Sancesario, G., Bernardini, S., De Angelis, G., Martino, G., Furlan, R., Centonze, D., 2014. Tumor necrosis factor is elevated in progressive multiple sclerosis and causes excitotoxic neurodegeneration. Mult. Scler. 20, 304–312.

Saylor, D., Dickens, A.M., Sacktor, N., Haughey, N., Slusher, B., Pletnikov, M., Mankowski, J.L., Brown, A., Volsky, D.J., McArthur, J.C., 2016. HIV-associated neurocognitive disorder—pathogenesis and prospects for treatment. Nat. Rev. Neurol. 12, 234–248.

Selmaj, K.W., Raine, C.S., 1988. Tumor necrosis factor mediates myelin and oligodendrocyte damage in vitro. Ann. Neurol. 23, 339–346.

Sevigny, J.J., Albert, S.M., McDermott, M.P., McArthur, J.C., Sacktor, N., Conant, K., Schifitto, G., Selnes, O.A., Stern, Y., McClernon, D.R., Palumbo, D., Kieburtz, K., Riggs, G., Cohen, B., Epstein, L.G., Marder, K., 2004. Evaluation of HIV RNA and markers of immune activation as predictors of HIV-associated dementia. Neurology 63, 2084–2090.

Wada, N.I., Jacobson, L.P., Margolick, J.B., Breen, E.C., Macatangay, B., Penugonda, S., Martinez-Maza, O., Bream, J.H., 2015. The effect of HAART-induced HIV suppression on circulating markers of inflammation and immune activation. AIDS 29, 463–471.

Woods, S.P., Morgan, E.E., Marquie-Beck, J., Carey, C.L., Grant, I., Letendre, S.L., Group, H.I.V.N.R.C, 2006. Markers of macrophage activation and axonal injury are associated with prospective memory in HIV-1 disease. Cogn. Behav. Neurol. 19, 217–221.

Wu, W., Guan, Y., Zhao, G., Fu, X.J., Guo, T.Z., Liu, Y.T., Ren, X.L., Wang, W., Liu, H.R., Li, Y.Q., 2016. Elevated IL-6 and TNF-alpha levels in cerebrospinal fluid of subarachnoid hemorrhage patients. Mol. Neurobiol. 53, 3277–3285.

Yajima, N., Kasama, T., Isozaki, T., Odai, T., Matsunawa, M., Negishi, M., Ide, H., Kameoka, Y., Hirohata, S., Adachi, M., 2005. Elevated levels of soluble fractalkine in active systemic lupus erythematosus: potential involvement in neuropsychiatric manifestations. Arthritis Rheum. 52, 1670–1675.