UC Davis

UC Davis Previously Published Works

Title

Serum MicroRNAs in HIV-Infected Individuals as Pre-Diagnosis Biomarkers for AIDS-NHL

Permalink

https://escholarship.org/uc/item/0gr8q63b

Journal

JAIDS Journal of Acquired Immune Deficiency Syndromes, 66(2)

ISSN

1525-4135

Authors

Thapa, Dharma R

Hussain, Shehnaz K

Tran, Wen-Ching

et al.

Publication Date

2014-06-01

DOI

10.1097/qai.0000000000000146

Peer reviewed

eScholarship.org Powered by the California Digital Library
University of California

NIH Public Access
Author Manuscript
J Acquir Immune Defic Syndr. Author manuscript; available in PMC 2015 June 01.

Published in final edited form as:
J Acquir Immune Defic Syndr. 2014 June 1; 66(2): 229–237. doi:10.1097/QAI.0000000000000146.

Serum microRNAs in HIV-infected individuals as pre-diagnosis biomarkers for AIDS-related non-Hodgkin lymphomas (AIDS-NHL)

Dharma R. Thapa, PhD[1,3,4], **Shehnaz K. Hussain, PhD**[2,5], **Wen-Ching Tran, BS**[4], **Gypsyamber D'souza, PhD**[6], **Jay H. Bream, PhD**[7], **Chad J. Achenback, MD**[8], **Velpandi Ayyavoo, PhD**[9], **Roger Detels, MD**[5], and **Otoniel Martínez-Maza, PhD**[1,2,3,4,5]

[1]UCLA AIDS Institute, David Geffen School of Medicine at UCLA, Los Angeles, CA

[2]Jonsson Comprehensive Cancer Center, David Geffen School of Medicine at UCLA, Los Angeles, CA

[3]Department of Obstetrics & Gynecology, David Geffen School of Medicine at UCLA, Los Angeles, CA

[4]Department of Microbiology, Immunology & Molecular Genetics, David Geffen School of Medicine at UCLA, Los Angeles, CA

[5]Department of Epidemiology, UCLA Fielding School of Public Health, Los Angeles, CA

[6]Department of Epidemiology, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD

[7]Department of Molecular Microbiology and Immunology, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD

[8]Northwestern University Feinberg School of Medicine, Chicago, IL

[9]Department of Infectious Diseases and Microbiology, University of Pittsburgh Graduate School of Public Health, Pittsburgh, PA

Abstract

Objective—To determine if changes in levels of serum microRNAs were seen preceding the diagnosis of AIDS-associated non-Hodgkin lymphoma (AIDS-NHL).

Design—Serum microRNA levels were compared in three subject groups from the Multicenter AIDS Cohort Study: HIV negative men (n=43), HIV positive men who did not develop NHL (n=45), and HIV positive men prior to AIDS-NHL diagnosis (n=62, median time prior to diagnosis, 8.8 months).

Correspondence: Otoniel Martinez-Maza PhD, UCLA AIDS Institute, 173 BSRB, 615 Charles E. Young Dr. South, Los Angeles, CA 90095-7363, Phone: (310) 825 2542, Fax: (310) 206 5387, omartinez@mednet.ucla.edu.

This work was presented at the 20th Conference on Retroviruses and Opportunistic Infections (CROI), March 2013, Atlanta, GA.

Conflicts of Interest

There are no conflicts of interest.

Conceived and designed the study: DRT, OM-M; performed the experiments: DRT, W-CT; analyzed data: DRT, SKH, OM-M; provided access to MACS specimens, contributed to discussion, and revision of manuscript: SKH, GD, JHB, CJA, VA, RD; wrote the paper: DRT, OM-M.

NIH-PA Author Manuscript
NIH-PA Author Manuscript
NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

Methods—A total of 175 serum-enriched microRNAs were initially screened to identify differentially expressed microRNAs among these groups, and the results validated by qPCR. Receiver-operating characteristic (ROC) analysis was then performed to assess biomarker utility.

Results—Higher levels of miR-21 and miR-122, and a lower level of miR-223, were able to discriminate HIV-infected from the HIV-uninfected groups, suggesting that these microRNAs are biomarkers for HIV infection, but are not AIDS-NHL specific. Among the HIV-infected groups, a higher level of miR-222 was able to discriminate diffuse large B-cell lymphoma (DLBCL) and primary central nervous system lymphoma (PCNSL) subjects from HIV-infected subjects who did not develop NHL, with area under the ROC curve of 0.777 and 0.792, respectively. At miR-222 cut-off values of 0.105 for DLBCL and 0.109 for PCNSL, the sensitivity and specificity were 75% and 77%; and 80% and 82%, respectively.

Conclusions—Altered serum levels of miR-21, miR-122, and miR-223 are seen in HIV-infected individuals. Higher serum level of miR-222 has clear potential as a serum biomarker for earlier detection of DLBCL and PCNSL among HIV-infected individuals.

Keywords

HIV; lymphoma; miR-21; miR-223; miR-122; miR-222

Introduction

The risk for non-Hodgkin lymphoma (NHL) is increased among HIV-infected (HIV+) individuals[1,2]. AIDS-related NHL (AIDS-NHL) are typically of B cell origin and include primary central nervous system lymphoma (PCNSL), as well as systemic lymphomas, including Burkitt lymphoma (BL) and diffuse large B-cell lymphoma (DLBCL)[3]. Despite a decrease in the incidence of NHL in the era of combination antiretroviral therapy (cART)[4–6], HIV+ individuals continue to be at increased risk for NHL[7,8]. In fact, in cART treated populations, NHL is the most frequent AIDS-defining cancer[9], with PCNSL and systemic NHL accounting for 10% and 29% of AIDS-related causes of death, respectively[10].

In recent years, microRNAs (miRNA) have been shown to have critical roles in lymphoma pathogenesis, and may serve as novel biomarkers for diagnosis or prognosis (reviewed in[11]). miRNAs are small non-coding RNAs that bind to the 3' untranslated region of messenger RNAs causing translational inhibition[12]. Numerous studies have identified the miRNA signatures of various B cell cancers[13–18], and have been used to distinguish BL from chronic lymphocytic leukemia (CLL)[19,20] or DLBCL[15], DLBCL from PCNSL[21] or follicular lymphoma[22–24], or between DLBCL subsets[25–28]. Tumor associated miRNAs have also been reported in serum of DLBCL[29,30] and CLL patients[31], cerebrospinal fluid (CSF) of PCNSL patients[32], and in blood B cells of patients who later develop AIDS-NHL[33]. In CLL, several miRNAs have been identified for risk stratification[34,35,36], associated with disease progression[37], and treatment-free and overall survival[37,38] Furthermore, in DLBCL patients undergoing immunochemotherapy, several miRNAs, including, miR-21[25,29], miR-222[27,39], miR-18a and miR-181a[39], a 8-miRNA signature[22], and a 9-miRNA signature[40], have been shown to predict survival. However, studies on miRNAs as biomarkers for early detection of NHL are lacking.

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

The availability of banked specimens from the Multicenter AIDS Cohort Study (MACS) has allowed us to assess pre-NHL diagnosis protein biomarkers[41–45]. Recently, we reported higher levels of miR-21 in circulating B cells up to three years prior to AIDS-NHL diagnosis[33]. However, serum miRNAs as early biomarkers for AIDS-NHL have not been explored, although, a recent study by Witwer *et al*. using a macaque/simian immunodeficiency virus (SIV) model showed differential levels of plasma miRNAs during acute infection, and a 6-miRNA signature that predicted CNS disease[46]. We hypothesized that serum miRNAs are dysregulated in HIV-infection, and that miRNAs are biomarkers for NHL. We screened the expression of 175 serum enriched miRNAs in three subject groups from the MACS, HIV negative men (HIV−), HIV positive men who did not develop NHL (HIV+), and HIV positive men who later developed NHL (pre-AIDS-NHL), to identify differentially expressed miRNAs, followed by verification using quantitative PCR (qPCR) assay.

Methods

Study population, tissues, and ethics statement

Serum samples from HIV negative men (HIV−, n=43), HIV+ men who did not develop NHL (HIV+, n=45), and HIV+ men who later developed NHL (pre-AIDS-NHL, n=62), were obtained from the MACS repository. For pre-AIDS-NHL cases, the median time prior to NHL diagnosis at the time of serum collection was 8.8 months, ranging from 2.5 to 21.5 months. The pre-AIDS-NHL group included three NHL subtypes: BL (n=10), DLBCL (n=32), and PCNSL (n=20). All samples, except two, were obtained from the pre-cART use era. The MACS is a prospective cohort study of gay/bisexual men (www.statepi.jhsph.edu/macs).[47]. Pertinent data, as well as peripheral blood mononuclear cells (PBMC) and serum/plasma, are collected at each semiannual visit. Information on hepatitis B virus (HBV) and hepatitis C virus (HCV) co-infection, as well as Epstein-Barr virus (EBV) tumor infection status for many of the NHL cases, was provided by the MACS. The MACS study protocols have been approved by the Institutional Review Board (IRB) at each MACS center. Anonymized tonsils were obtained from the UCLA Translational Pathology Core Laboratory (TPCL). AIDS-related tumors were obtained from the AIDS & Cancer Specimen Resource (ACSR).

RNA extraction

Serum RNA was extracted using TRIzol LS reagent from Life Technologies (Carlsbad, USA). Each 250uL of serum was extracted with 750uL of TRIzol LS (this ratio was always maintained) per the manufacturer's protocol. 40ug of glycogen (Life Technologies, Carlsbad, USA) was added to the aqueous phase to aid in nucleic acid precipitation. RNA was resuspended in 25μL of water and quantified using Quant-iT™ RiboGreen RNA Reagent and Kit (Molecular Probes, Eugene, USA). The average yield (±SD) of total RNA was 102 ±27 ng per 1ml of serum. For tonsillar B cells, tonsils were first minced and the mononuclear cells were separated using Ficoll-paque (GE Healthcare, Uppsala, Sweden). B cells were separated from the mononuclear population using CD19+ magnetic beads (Life Technologies, USA). B cells from PBMC obtained from the MACS were also separated in a

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

similar manner. Total RNA from these B cells and tumor specimens were extracted using the mirVana miRNA isolation kit (Ambion, Austin, USA).

microRNA screening to identify differentially expressed microRNAs

Exiqon's Serum/Plasma Focus microRNA PCR panels (Exiqon, Vedbaek, Denmark) was used to identify differentially expressed serum microRNAs between HIV− (n=8), HIV+ (n=7), and pre-AIDS-NHL (n=7) subject groups. This panel consists of two 96-well PCR plates containing a total of 175 pre-defined serum/plasma enriched miRNAs with additional seven miRNAs (miR-451, miR-16, miR-103, miR-425, miR-423-5p, miR-93, miR-191) as potential references. First, cDNA synthesis was performed using miRCURY LNA™ Universal RT microRNA PCR kit (Exiqon, Vedbaek, Denmark). Each reaction consisted of 4μL of 5× reaction buffer, 2μL of enzyme mix, 1μL synthetic spike, 4ng total RNA, and water to a final volume of 20μL. This mixture was incubated for 1hr at 42°C, 5min at 95°C, and stored at −20°C. For real-time PCR amplification, the cDNA was diluted 50× with water and then mixed 1:1 with 2μ SYBR® Green master mix (Exiqon, Vedbaek, Denmark). 10μL aliquot of this mixture was dispensed to each well of 96-well PCR panels. Real-time PCR was performed using ABI 7300 real-time machine (Applied Biosystems, Foster City, USA) using the following protocol: 95°C for 1min and 40 cycles of 95°C for 10s and 60°C for 1min. RNA spike-in control (UniSp6 CP) added during the RT step was used to monitor optimal cDNA synthesis across samples. Readings between plates were normalized using inter-plate calibrators (UniSp3). Spurious amplifications were monitored using the no-template control wells. Threshold cycles (Ct) were obtained and levels of all miRNAs were normalized to miR-16 using $dCt=Ct_{miRNA}-Ct_{miR\text{-}16}$. Differentially expressed miRNAs was identified using significance analysis of microarrays (SAM) feature of the MultiExperiment Viewer Software v4.8 (http://mev.tm4.org)[48] with false discovery rate (FDR) set to 0%.

Quantitative real-time PCR (qRT-PCR)

Individual miRNAs were quantified using TaqMan® MicroRNA Reverse Transcription Kit and TaqMan® MicroRNA Assay kits (Applied Biosystems, Foster City, USA). Briefly, total RNA was reverse-transcribed in a reaction mix containing 1.5μL of 10× RT buffer, 0.19μL of RNase inhibitor, 0.15μL of dNTP mix, 5μL of 3× primer mix (of 11 miRNAs), 5μL RNA (equivalent RNA in ~50uL serum volume), 1.0μL of Multiscribe™ RT enzyme, and H_20 to a final volume of 15μL; and subjected to the following thermal protocol: 30 min at 16°C; 30 min at 42°C; and hold at 4°C. The RT product was then diluted to 225uL. Each qPCR reaction consisted of 10μL of Taqman® 2× Universal PCR Master Mix (No AmpErase® UNG), 1μL of 20× microRNA specific assay primers/probe mixture, 5μL of the RT product, and H_2O to a final volume of to 20μL. qPCR was performed using ABI 7300 qPCR machine with the following protocol: 10 min at 95°C followed by 40 cycles of 15 sec at 95°C (denature) and 60 sec at 60°C (anneal/extend). Each sample was assayed in triplicates. The level of all serum miRNAs were normalized to miR-16 (to be consistent with the screening protocol), whereas cellular miRNAs were normalized to RNU 48 (small nucleolar RNA), using the following expression, $dCt=Ct_{miRNA}-Ct_{miR\text{-}16(or\ RNU48)}$. The relative expression of miRNAs was calculated using 2^{-dCt}.

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

Statistical Analysis

Groupwise miRNA levels were compared using Kruskal-Wallis test with multiple comparison test as indicated. Receiver-operating characteristics (ROC) curves were generated to assess the diagnostic value of serum miRNAs to discriminate between HIV−, HIV+, and pre-AIDS-NHL groups. $p<0.05$ was considered statistically significant. All analysis was performed using GraphPad Prism 5.0 software (La Jolla, USA).

Results

Subject characteristics

Characteristics of the subject population are described in Table 1. Although there was no significant difference in the median age of subjects in the HIV−, HIV, and pre-AIDS-NHL groups (43.4, 36.4, and 40.2 years, respectively) (p=0.064), the HIV− group was about 5 years older than the HIV+ groups. As expected, there were lower numbers of CD4+ cells in the HIV+ group compared to the HIV− group (424 vs 995 cells/μL), and of the pre-AIDS-NHL group compared to the HIV+ group (160 vs 424 cells/μL) ($p<0.001$, both comparisons). There was no significant difference in the duration of HIV positivity between the HIV+ and pre-AIDS-NHL groups (5.2 vs 5.5 years) (p=0.672).

Screening for differentially expressed serum miRNAs

Initial screening for differentially expressed serum miRNAs was performed using the Exiqon Serum Focus microRNA PCR Panel, utilizing a subset of samples from the three subject groups (see methods). The raw Ct values obtained for all 175 miRNAs is attached as supplemental data 1. The average C_t (± SD) for the highest expressing miR-451 was 25.03±1.99 and the lowest expressing miR-182 was 37.65±1.20. miR-16 levels were 28.13±1.94, and was used as our reference. Heatmap of 10 miRNAs identified by SAM analysis (at FDR of 0%) as being differentially expressed is shown (Fig.1)

Validation of screening results by qPCR

Validation of screening results was performed by qPCR quantification of miRNAs from our entire sample set which included the HIV− group (n=43), HIV+ group (n=45), and the pre-AIDS-NHL group (n=62), which consisted of lymphoma subtypes: BL (n=10), DLBCL (n=32), and PCNSL (n=20). Out of the 10 miRNAs identified in our screening, four miRNAs (miR-22, miR-99a, miR-22*, miR-29a) were detected at low levels and two miRNAs (miR-29c, miR-130a) showed no significant differences in the larger group. In order to increase confidence in our findings, these miRNAs were excluded from further analysis. The differential expression of the remaining miRNAs (miR-21, miR-223, miR-122, and miR-222) was validated by qPCR (Fig. 2a–d).

Compared to the HIV− group, HIV+ group had a significantly higher median level of miR-21 (0.051 vs 0.105, $p<0.001$) and miR-122 (0.012 vs 0.034, $p<0.01$), and a lower level of miR-223 (1.131 vs 0.501, $p<0.001$) (Fig. 2a–c). Likewise, compared to the HIV− group, there was a higher level of miR-21 seen in the BL, DLBCL, and PCNSL subgroups (0.051 vs 0.151, 0.160, 0.146 respectively, $p<0.01$ for all), higher miR-122 levels in DLBCL and PCNSL subgroups (0.012 vs 0.029, 0.043 respectively, $p<0.001$ for both), higher miR-222

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

levels in the DLBCL and PCNSL subgroups (0.046 vs 0.127, 0.150 respectively, $p<0.001$ for both), and a lower level of miR-223 in the DLBCL and PCNSL subgroups (1.131 vs 0.634, 0.712 respectively, $p<0.05$ for both) (Fig. 2a–d). Only miR-222 levels were seen to be significantly different between the HIV+ and pre-AIDS-NHL groups. Compared to the HIV + group, miR-222 expression was higher in the DLBCL and PCNSL subgroups (0.063 vs 0.127, 0.150 respectively, $p<0.001$ for both) (Fig. 2d). Because CD4 counts varied between the subject groups, we assessed whether levels of miR-222 was correlated with CD4 counts. We found no such correlation within the HIV+ group, which had a wide range of CD4 levels ($r=-0.133$, $P=0.389$) (Fig. 2e).

Serum miRNAs as potential biomarkers for HIV infection and AIDS-NHL diagnosis

The ability of miRNAs (miR-21, miR-223, and miR-122) to discriminate between HIV− and HIV+ groups was demonstrated with ROC plot analysis yielding area under curves (AUC) of 0.773 (95% CI: 0.673–0.873), 0.804 (95% CI: 0.712–0.897), and 0.726 (95% CI: 0.662–0.830) respectively ($p<0.0001$, for all) (Fig. 3a). These same miRNAs were also able to discriminate between HIV− and pre-AIDS-NHL groups with an AUC of 0.876 (95% CI: 0.804–0.948) for miR-21, 0.740 (95% CI: 0.643–0.836) for miR-223, and 0.777 (95% CI: 0.686–0.868) for miR-122, ($p<0.001$, for all) (Fig. 3b). miR-222 could not distinguish HIV− from HIV+ groups [AUC: 0.588 (95% CI: 0.468–0.707), $p=0.154$], but could discriminate HIV− from pre-AIDS-NHL groups [AUC: 0.848 (95% CI: 0.773–0.922), $p<0.0001$] (Fig. 3a,b). A separate ROC analysis of miR-222 showed that it was able to discriminate HIV+ group from the DLBCL group [AUC: 0.777 (95% CI: 0.670–0.884)] and PCNSL group [AUC: 0.792 (95% CI: 0.663–0.920)] ($p<0.001$, for both), but not the BL group [AUC: 0.624 (95% CI: 0.469–0.779), $p=0.221$] (Fig. 3c). At cut-off values of 0.105 for DLBCL and 0.109 for PCNSL, miR-222 levels were predictive of NHL diagnosis in HIV+ subjects with a sensitivity and specificity of 75% and 77%, and 80% and 82%, respectively. Overall, a classification tree based on cut-off values of miR-21, miR-223, and miR-122 correctly distinguished all HIV-infected subjects but one, from HIV-uninfected subjects (99%), while higher levels of miR-222 identified up to 85% of PCNSL and 78.1% of DLBCL from among the HIV-infected subjects (Fig. 3d).

miR-222 levels are elevated in AIDS-DLBCL and AIDS-PCNSL tumors

We explored possible cellular sources of serum miR-222 that may contribute to the higher levels seen preceding AIDS-NHL diagnosis. First, we examined the levels of miR-222 in circulating blood cells, finding that levels of miR-222 were not significantly different in the B cell or the non-B cell fraction (B cell depleted PBMC) among HIV−, HIV+, or pre-AIDS-NHL subjects, suggesting a non-blood cell source for miR-222 (Fig. 4). Consequently, we examined miR-222 levels in various primary AIDS-NHL tumors and tonsillar B cells. Compared to the overall level in blood cells or tonsillar B cells, miR-222 expression was significantly higher in DLBCL ($p<0.01$) and PCNSL tumors ($p<0.001$) but not in BL tumors ($p=0.121$) (Fig. 4). This trend is in agreement with our results on serum miR-222 levels among the NHL subtypes (Fig. 2d).

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

Association of serum miRNA levels and HBV, HCV and EBV infection status

We also looked at the association of miR-21, miR-223, miR-122, and miR-222 levels with the HBV, and HCV status of our study subjects, as well as with the EBV status of the NHLs. Among pre-NHL subjects, no significant association of these miRNAs with EBV status was seen (Fig S1). Among all HIV infected subjects, higher miR-122 and miR-21 was associated with HBV infection (Fig S2), whereas none of these miRNAs were associated with HCV status (Fig S3). Overall, these results suggest serum miR-222 to be a predictive biomarker for NHL subsets irrespective of tumor EBV status, while HBV infection is associated with elevated serum levels of miR-21 and the liver enriched miR-122.

Discussion

Circulating miRNAs have shown promise as cancer biomarkers[49,50]. However, studies exploring serum miRNAs as pre-cancer diagnosis biomarkers in high-risk populations are lacking. In this study, we demonstrate that levels of serum miRNAs are altered in HIV-infected individuals. Three miRNAs (miR-21, miR-223, miR-122) were able to discriminate HIV+ from uninfected subjects. However, these miRNAs were not able to discriminate HIV + subjects who did not develop NHL from those who did, suggesting that HIV-associated changes, rather than nascent NHL tumors, contributed to their dysregulation. We did, however, find levels of miR-222 were able to discriminate HIV-infected subjects who did not develop NHL (HIV+ group) from those who later developed DLBCL or PCNSL, with a sensitivity and specificity of 75% and 77%, and 80% and 82%, respectively. We further showed that miR-222 was overexpressed in primary DLBCL or PCNSL tumors.

Several questions remain regarding the significance of miRNAs in blood. An altered PBMC miRNA profile has been observed in HIV infection[51,52], CD4+ T cells[53], Jurkat cells[54], HeLa cell lines[55], PBMC of elite suppressors compared to viremic patients[56], and CD4+ T cells of elite long-term nonprogressors compared to multiply exposed uninfected patients[53]. Studies have also implicated miRNAs in host defense against HIV, such as in the susceptibility of monocytes/macrophages to infection[57], maintaining latency in infected T cells[58], and suppression of viral replication[54,59]. In a SIV model, a 45-miRNA plasma signature (including, higher miR-21, miR-222; and lower miR-223, as was seen by us) was associated with acute infection[46]. In summary, the complex interaction of host and virus involves miRNAs, and together might explain dysregulation of serum miRNAs in HIV-infected individuals.

The lower level of serum miR-223 that we see in HIV-infected subject groups is likely the result of lymphocyte activation in the milieu of chronic immune stimulatory environment. miR-223 is expressed at lower levels in activated T cells[58], activated germinal center B cells[20,27,60], and in *in vitro* IL4/CD40-stimulated B cells (unpublished data). Lower serum miR-223 (and miR-146a) has also been observed in patients with systemic inflammatory response syndrome, with a further significant decrease seen in patients with sepsis[61]. Also, given that variations within blood cell subpopulations can alter serum miRNA levels[62], lower serum miR-223 level may also be associated with age, or with the decline in the number of miR-223-rich cells such as neutrophils[63,64] or T lymphocytes seen during HIV

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

infection, although we did not find a significant correlation with CD4+ T cell subsets (data not shown).

miR-122 is a liver-specific miRNA[65] and is not associated with normal B-cell biology or tumorigenesis. Several HIV-related factors, such as coinfection with HCV or HBV, liver toxicity from cART, and alcohol abuse, among others[66], has made liver disease the most common non-AIDS-related cause of death among HIV-infected persons[67]. Numerous studies have implicated miR-122 in liver diseases, such as in hepatocellular carcinoma[68–70], chronic hepatitis[68], HCV-induced fibrosis[71], or drug-induced liver injury[72].

The potential association of these four serum miRNAs with HBV or HCV status, or with tumor EBV status, was examined. No significant association of these miRNAs with EBV status was seen (Fig S1). Among HIV+ subjects, higher miR-122 and miR-21 were associated with HBV infection (Fig S2), whereas none of these miRNAs were associated with HCV infection (Fig S3). These results suggest that serum miR-222 is a predictive biomarker for AIDS-NHL, irrespective of EBV status. The association between chronic HBV infection and elevated miR-21 and miR-122 levels suggests that liver infection may result in elevated serum levels of these miRNAs.

The miRNAs identified in our study have been reported as being useful biomarkers for B cell malignancies. Higher miR-21 levels in PBMC correlated with lower overall survival in CLL, higher levels in CSF was diagnostic of PCNSL[32], higher serum levels correlated with longer relapse-free survival in DLBCL patients[25,29], and higher plasma levels were predictive of CNS disease in a SIV model[46]. Here, we saw increased serum miR-21 in both the HIV+ and pre-AIDS-NHL groups, compared to the HIV− group, although the levels were not higher in the pre-AIDS-NHL group compared to the HIV+ group. Thus, given the increased levels in HIV+ individuals, serum miR-21 may not be a biomarker for early detection of AIDS-NHL, although miR-21 levels obtained at diagnosis might have prognostic value. The design of our study is in contrast to that of previous studies, where serum miRNA levels were measured at, or post DLBCL diagnosis and compared to healthy controls[29,30]. Additionally, miR-21 is overexpressed in a wide variety of other cancers types (both in tissues and serum)[73,74], making it a diagnostic biomarker with good sensitivity but poor specificity. This feature makes miR-21 undesirable for use as an early diagnostic marker for NHL in HIV-infected individuals, as they are at increased risk not only for NHL but for other non-AIDS defining cancers as well[10,75].

Only miR-222 was able to discriminate between HIV-infected subjects who did or did not develop NHL, suggesting a role for this miRNA in B cell malignancies. In support of this, several studies in the non-HIV setting have implicated B cell tumor expressed miR-222 as a prognostic marker. For example, in DLBCL patients undergoing immunochemotherapy, higher miR-222 level correlated with poorer overall survival[27,40] or progression-free survival[27,39,40], either as a sole biomarker[27], or in combination with other miRNAs[39,40]. In CLL, higher miR-222 levels were seen in plasma[31], with higher cellular miR-222 levels associated with disease progression[36], and resistance to fludarabine treatment[76].

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

It is notable that we found higher levels of miR-222 in pre-diagnosis DLBCL and PCNSL, but not BL serum or tumor tissues. PCNSL are DLBCL with immunoblastic features presenting in the CNS[3]. Despite the molecular heterogeneity of DLBCLs, it retains a miRNA signature distinct from BL[13,15] including higher miR-222 level in DLBCL compared to BL[15]. Additionally, we found no difference in the level of miR-222 in circulating cells, either in the B cell, or the non-B cell compartment, among the three subject groups. Given these findings, and the relative proximity to the time of diagnosis that our samples were obtained (median of 8.8 months pre-diagnosis), it is probable that the cellular source for serum miR-222 preceding NHL diagnosis is premalignant cells and/or as yet undiagnosed DLBCL.

In conclusion, serum miRNAs are promising biomarkers for early detection of AIDS-NHL. Future expanded studies, including work with other cohorts, in conjunction with global miRNA screening, will be needed for the identification and verification of circulating miRNAs as bona fide biomarkers for NHL.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We would like to thank Larry Magpantay, Jose Merino, and Lupita Pena from the Martinez-Maza lab for their support; Christopher Coley and Janet Schollenberger for providing the relevant laboratory data on the MACS subjects; and all the MACS participants whose extraordinary commitment and dedication over the years has made studies like this possible.

Sources of Funding:

This work was supported by grants from the Margaret E. Early Trust (B-MEEMR 20113120) and the National Institutes of Health (R01-CA-168482, R01-CA-057152, NCI supplements to U01-AI-035040, and K07-CA-140360 to SKH). The Multicenter AIDS Cohort Study (MACS) includes the following: Baltimore: Baltimore: The Johns Hopkins University Bloomberg School of Public Health: Joseph B. Margolick (PI), Barbara Crain, Adrian Dobs, Homayoon Farzadegan, Joel Gallant, Lisette Johnson-Hill, Michael W. Plankey, Ned Sacktor, Ola Selnes, James Shepard, Chloe Thio. Chicago: Feinberg School of Medicine, Northwestern University, and Cook County Bureau of Health Services: Steven M. Wolinsky (PI), John P. Phair, Sheila Badri, Maurice O'Gorman, David Ostrow, Frank Palella, Ann Ragin. Los Angeles: University of California, UCLA Schools of Public Health and Medicine: Roger Detels (PI), Otoniel Martínez-Maza (Co-P I), Aaron Aronow, Robert Bolan, Elizabeth Breen, Anthony Butch, Beth Jamieson, Eric N. Miller, John Oishi, Harry Vinters, Dorothy Wiley, Mallory Witt, Otto Yang, Stephen Young, Zuo Feng Zhang. Pittsburgh: University of Pittsburgh, Graduate School of Public Health: Charles R. Rinaldo (PI), Lawrence A. Kingsley (Co-PI), James T. Becker, Ross D. Cranston, Jeremy J. Martinson, John W. Mellors, Anthony J. Silvestre, Ronald D. Stall. Data Coordinating Center: The Johns Hopkins University Bloomberg School of Public Health: Lisa P. Jacobson (PI), Alvaro Munoz (Co-PI), Alison, Abraham, Keri Althoff, Christopher Cox, Gypsyamber D'Souza, Priya Duggal, Elizabeth Golub, Janet Schollenberger, Eric C. Seaberg, Sol Su, Pamela Surkan. NIH: National Institute of Allergy and Infectious Diseases: Robin E. Huebner; National Cancer Institute: Geraldina Dominguez. UO1-AI-35042, UM1-AI-35043, UO1-AI-35039, UO1-AI-35040, UO1-AI-35041, and UL1-RR025005 (NCATS). Website located at http://www.statepi.jhsph.edu/macs/macs.html.

References

1. Cote TR, Biggar RJ, Rosenberg PS, et al. Non-Hodgkin's lymphoma among people with AIDS: incidence, presentation and public health burden. Int J Cancer. 1997; 73(5):645–650. [PubMed: 9398040]
2. Beral V, Peterman T, Berkelman R, Jaffe H. AIDS-associated non-Hodgkin lymphoma. Lancet. 1991 Apr 6; 337(8745):805–809. [PubMed: 1672911]

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

3. Raphael, M.; Borisch, B.; Jaffe, ES. Lymphomas associated with infection by the human immune deficiency virus (HIV). In: Jaffe, ES.; Harris, NL.; Stein, H.; Vardiman, JW., editors. World Health Organization Classification of Tumors, Pathology and Genetics of Tumours of Haemotopoietic and Lymphoid Tissues. Lyon: IARC Press; 2001. p. 260-263.
4. Bohlius J, Schmidlin K, Costagliola D, et al. Incidence and risk factors of HIV-related non-Hodgkin's lymphoma in the era of combination antiretroviral therapy: a European multicohort study. Antivir Ther. 2009; 14(8):1065–1074. [PubMed: 20032536]
5. Diamond C, Taylor TH, Aboumrad T, Anton-Culver H. Changes in acquired immunodeficiency syndrome-related non-Hodgkin lymphoma in the era of highly active antiretroviral therapy: incidence, presentation, treatment, and survival. Cancer. 2006 Jan 1; 106(1):128–135. [PubMed: 16329140]
6. Long JL, Engels EA, Moore RD, Gebo KA. Incidence and outcomes of malignancy in the HAART era in an urban cohort of HIV-infected individuals. AIDS. 2008 Feb 19; 22(4):489–496. [PubMed: 18301061]
7. Seaberg EC, Wiley D, Martinez-Maza O, et al. Cancer incidence in the multicenter AIDS Cohort Study before and during the HAART era: 1984 to 2007. Cancer. 2010 Dec 1; 116(23):5507–5516. [PubMed: 20672354]
8. Silverberg MJ, Chao C, Leyden WA, et al. HIV infection, immunodeficiency, viral replication, and the risk of cancer. Cancer Epidemiol Biomarkers Prev. 2011 Dec; 20(12):2551–2559. [PubMed: 22109347]
9. d'Arminio Monforte A, Sabin CA, Phillips A, et al. The changing incidence of AIDS events in patients receiving highly active antiretroviral therapy. Arch Intern Med. 2005 Feb 28; 165(4):416–423. [PubMed: 15738371]
10. Bonnet F, Lewden C, May T, et al. Malignancy-related causes of death in human immunodeficiency virus-infected patients in the era of highly active antiretroviral therapy. Cancer. 2004 Jul 15; 101(2):317–324. [PubMed: 15241829]
11. Di Lisio L, Martinez N, Montes-Moreno S, Piris-Villaespesa M, Sanchez-Beato M, Piris MA. The role of miRNAs in the pathogenesis and diagnosis of B-cell lymphomas. Blood. 2012 Aug 30; 120(9):1782–1790. [PubMed: 22760782]
12. Bartel DP. MicroRNAs:genomics, biogenesis, mechanism, and function. Cell. 2004; 116(2):281–297. [PubMed: 14744438]
13. Di Lisio L, Sanchez-Beato M, Gomez-Lopez G, et al. MicroRNA signatures in B-cell lymphomas. Blood Cancer J. 2012; 2(2):e57. [PubMed: 22829247]
14. Jima DD, Zhang J, Jacobs C, et al. Deep sequencing of the small RNA transcriptome of normal and malignant human B cells identifies hundreds of novel microRNAs. Blood. 2010 Dec 2; 116(23):e118–e127. [PubMed: 20733160]
15. Lenze D, Leoncini L, Hummel M, et al. The different epidemiologic subtypes of Burkitt lymphoma share a homogenous micro RNA profile distinct from diffuse large B-cell lymphoma. Leukemia. 2011; 25(12):1869–1876. [PubMed: 21701491]
16. O'Hara AJ, Wolfgang V, Dittmer DP. Gene alteration and precursor and mature microRNA transcription changes contribute to the miRNA signature of primary effusion lymphoma. Blood. 2008; 111(4):2347–2353. [PubMed: 18079361]
17. Navarro A, Gaya A, Martinez A, et al. MicroRNA expression profiling in classic Hodgkin lymphoma. Blood. 2008; 111(5):2825–2832. [PubMed: 18089852]
18. Calin GA, Chang-Gong L, Sevignani C, et al. MicroRNA profiling reveals distinct signatures in B cell chronic lymphocytic leukemias. Proc Natl Acad Sci USA. 2004; 101(32):11755–11760. [PubMed: 15284443]
19. Robertus JL, Kluiver J, Weggemans C, et al. MiRNA profiling in B non-Hodgkin lymphoma: a MYC-related miRNA profile characterizes Burkitt lymphoma. Br J Haematol. 2010 Jun; 149(6): 896–899. [PubMed: 20331457]
20. Zhang J, Jima DD, Jacobs C, et al. Patterns of microRNA expression characterize stages of human B-cell differentiation. Blood. 2009; 113(19):4586–4594. [PubMed: 19202128]

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

21. Fischer L, Hummel M, Korfel A, Lenze D, Joehrens K, Thiel E. Differential micro-RNA expression in primary CNS and nodal diffuse large B-cell lymphomas. Neuro Oncol. 2011; 13(10): 1090–9098. [PubMed: 21803762]
22. Lawrie CH, Chi J, Taylor S, et al. Expression of microRNAs in diffuse large B cell lymphoma is associated with immunophenotype, survival and transformation from follicular lymphoma. J Cell Mol Med. 2009; 13(7):1248–1260. [PubMed: 19413891]
23. Fassina A, Marino F, Siri M, et al. The miR-17-92 microRNA cluster: a novel diagnostic tool in large B-cell malignancies. Lab Invest. 2012 [Epub ahead of print].
24. Roehle A, Hoefig KP, Repsilber D, et al. MicroRNA signatures characterize diffuse large B-cell lymphomas and follicular lymphomas. Br J Haematol. 2008; 142(5):732–744. [PubMed: 18537969]
25. Lawrie CH, Soneji S, Marafioti T, et al. MicroRNA expression distinguishes between germinal center B cell-like and activated B cell-like subtypes of diffuse large B cell lymphoma. Int J Cancer. 2007; 121(5):1156–1161. [PubMed: 17487835]
26. Culpin RE, Proctor SJ, Angus B, Crosier S, Anderson JJ, Mainou-Fowler T. A 9 series microRNA signature differentiates between germinal center and activated B-cell-like diffuse large B-cell lymphoma cell lines. Int J Oncol. 2010; 37:367–376. [PubMed: 20596664]
27. Malumbres R, Sarosiek KA, Cubedo E, et al. Differentiation-stage-specific expression of microRNAs in B-lymphocytes and diffuse B-cell lymphomas. Blood. 2009; 113(16):3754–3764. [PubMed: 19047678]
28. Li C, Sang-Woo K, Rai D, et al. Copy number abnormalities, MYC activity, and the genetic fingerprint of normal B cells mechanistically define the microRNA profile of diffuse large B-cell lymphoma. Blood. 2009; 113(26):6681–6690. [PubMed: 19278952]
29. Lawrie CH, Gal S, Dunlop HM, et al. Detection of elevated levels of tumor-associated microRNAs in serum of patients with diffuse large B-cell lymphoma. Br J Haematol. 2008; 141(5):672–675. [PubMed: 18318758]
30. Fang C, Zhu DX, Dong HJ, et al. Serum microRNAs are promising novel biomarkers for diffuse large B cell lymphoma. Ann Hematol. 2012; 91(4):553–559. [PubMed: 21987025]
31. Moussay E, Wang K, Cho JH, et al. MicroRNA as biomarkers and regulators in B-cell chronic lymphocytic leukemia. Proc Natl Acad Sci U S A. 2011 Apr 19; 108(16):6573–6578. [PubMed: 21460253]
32. Baraniskin A, Kuhnhenn J, Schlegel U, et al. Identification of microRNAs in the cerebrospinal fluid as marker for primary diffuse large B-cell lymphoma of the central nervous system. Blood. 2011; 117(11):3140–3146. [PubMed: 21200023]
33. Thapa DR, Bhatia K, Bream JH, et al. B-cell activation induced microRNA-21 is elevated in circulating B cells preceding the diagnosis of AIDS-related non-Hodgkin lymphomas. AIDS. 2012; 26(9):1177–1180. [PubMed: 22487708]
34. Stamatopoulos B, Meuleman N, Haibe-Kains B, et al. microRNA-29c and microRNA-223 down-regulation has in vivo significance in chronic lymphocytic leukemia and improves disease risk stratification. Blood. 2009; 113(21):5237–5245. [PubMed: 19144983]
35. Visone R, Veronese A, Rassenti LZ, et al. miR-181b is a biomarker of disease progression in chronic lymphocytic leukemia. Blood. 2011 Sep 15; 118(11):3072–3079. [PubMed: 21636858]
36. Calin GA, Ferracin M, Cimmino A, et al. A microRNA signature associated with prognosis and progression in chronic lymphocytic leukemia. N Engl J Med. 2005; 353(17):1793–1801. [PubMed: 16251535]
37. Asslaber D, Pinon JD, Seyfried I, et al. microRNA-34a expression correlates with MDM2 SNP309 polymorphism and treatment-free survival in chronic lymphocytic leukemia. Blood. 2010 May 27; 115(21):4191–4197. [PubMed: 20089965]
38. Rossi S, Shimizu M, Barbarotto E, et al. microRNA fingerprinting of CLL patients with chromosome 17p deletion identify a miR-21 score that stratifies early survival. Blood. 2010 Aug 12; 116(6):945–952. [PubMed: 20393129]
39. Alencar AJ, Malumbres R, Kozloski GA, et al. MicroRNAs are independent predictors of outcome in diffuse large B-cell lymphoma patients treated with R-CHOP. Clin Cancer Res. 2011; 17:4125–4135. [PubMed: 21525173]

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

40. Montes-Moreno S, Martinez N, Sanchez-Espiridion B, et al. miRNA expression in diffuse large B-cell lymphoma treated with chemoimmunotherapy. Blood. 2011; 118(4):1034–1040. [PubMed: 21633089]
41. Breen EC, Hussain SK, Magpantay L, et al. B-cell stimulatory cytokines and markers of immune activation are elevated several years prior to the diagnosis of systemic AIDS-associated non-Hodgkin B-cell lymphoma. Cancer Epidemiol Biomarkers Prev. 2011 Jul; 20(7):1303–1314. [PubMed: 21527584]
42. Epeldegui M, Breen EC, Hung YP, Boscardin WJ, Detels R, Martinez-Maza O. Elevated expression of activation induced cytidine deaminase in peripheral blood mononuclear cells precedes AIDS-NHL diagnosis. AIDS. 2007; 21:2265–2270. [PubMed: 18090274]
43. Pluda JM, Venzon DJ, Tosato G, et al. Parameters affecting the development of non- Hodgkin's lymphoma in patients with severe human immunodeficiency virus infection receiving antiretroviral therapy. J Clin Oncol. 1993 Jun; 11(6):1099–1107. [PubMed: 8099121]
44. Breen EC, Epeldegui M, Boscardin WJ, Widney DP, Detels R, Martinez-Maza O. Elevated levels of soluble CD44 precede the development of AIDS-associated non- Hodgkin's B-cell lymphoma. AIDS. 2005; 19(15):1711–1712. [PubMed: 16184051]
45. De Roos AJ, Mirick DK, Edlefsen KL, et al. Markers of B-cell activation in relation to risk of non-Hodgkin lymphoma. Cancer Res. 2012 Sep 15; 72(18):4733–4743. [PubMed: 22846913]
46. Witwer KW, Sarbanes SL, Liu J, Clements JE. A plasma microRNA signature of acute lentiviral infection: biomarkers of central nervous system disease. AIDS. 2011 Nov 13; 25(17):2057–2067. [PubMed: 21857495]
47. Kaslow RA, Ostrow DG, Detels R, Phair JP, Polk BF, Rinaldo CR Jr. The Multicenter AIDS Cohort Study: rationale, organization, and selected characteristics of the participants. Am J Epidemiol. 1987 Aug; 126(2):310–318. [PubMed: 3300281]
48. Saeed AI, Sharov V, White J, et al. TM4: a free, open-source system for microarray data management and analysis. Biotechniques. 2003; 34(2):374–378. [PubMed: 12613259]
49. Kosaka N, Iguchi H, Ochiya T. Circulating microRNA in body fluid: a new potential biomarker for cancer diagnosis and prognosis. Cancer Sci. 2010; 101(10):2087–2092. [PubMed: 20624164]
50. Allegra A, Alonci A, Campo S, et al. Circulating microRNAs: New biomarkers in diagnosis, prognosis and treatment of cancer (Review). Int J Oncol. 2012 Dec; 41(6):1897–1912. [PubMed: 23026890]
51. Houzet L, Yeung ML, de Lame V, Desai D, Smith SM, Jeang KT. MicroRNA profile changes in human immunodeficiency virus type 1 (HIV-1) seropositive individuals. Retrovirology. 2008; 5(118):118. [PubMed: 19114009]
52. Gupta A, Nagilla P, Le HS, et al. Comparative expression profile of miRNA and mRNA in primary peripheral blood mononuclear cells infected with human immunodeficiency virus (HIV-1). PLoS ONE. 2011; 6(7):e22730. [PubMed: 21829495]
53. Bignami F, Pilotti E, Bertoncelli L, et al. Stable changes in CD4+ T lymphocyte miRNA expression after exposure to HIV-1. Blood. 2012 Jun 28; 119(26):6259–6267. [PubMed: 22286198]
54. Triboulet R, Mari B, Lin YL, et al. Suppression of microRNA-silencing pathway by HIV-1 during virus replication. Science. 2007 Mar 16; 315(5818):1579–1582. [PubMed: 17322031]
55. Yeung ML, Bennasser Y, Myers TG, Jiang G, Benkirane M, Jeang KT. Changes in microRNA expression profiles in HIV-1-transfected human cells. Retrovirology. 2005; 2(81):81. [PubMed: 16381609]
56. Witwer KW, Watson AK, Blankson JN, Clements JE. Relationships of PBMC microRNA expression, plasma viral load, and CD4+ T-cell count in HIV-1-infected elite suppressors and viremic patients. Retrovirology. 2012; 9(5):5. [PubMed: 22240256]
57. Wang X, Ye L, Hou W, et al. Cellular microRNA expression correlates with susceptibility of monocytes/macrophages to HIV-1 infection. Blood. 2009 Jan 15; 113(3):671–674. [PubMed: 19015395]
58. Huang J, Wang F, Argyris E, et al. Cellular microRNAs contribute to HIV-1 latency in resting primary CD4+ T lymphocytes. Nat Med. 2007 Oct; 13(10):1241–1247. [PubMed: 17906637]

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

59. Ahluwalia JK, Khan SZ, Soni K, et al. Human cellular microRNA hsa-miR-29a interferes with viral nef protein expression and HIV-1 replication. Retrovirology. 2008; 5(117):117. [PubMed: 19102781]
60. Thapa DR, Li X, Jamieson BD, Martinez-Maza O. Overexpression of microRNAs from the miR-17-92 paralog clusters in AIDS-related non-Hodgkin's lymphomas. PLoS ONE. 2011; 6(6):e20781. [PubMed: 21698185]
61. Wang JF, Yu ML, Yu G, et al. Serum miR-146a and miR-223 as potential new biomarkers for sepsis. Biochem Biophys Res Commun. 2010 Mar 26; 394(1):184–188. [PubMed: 20188071]
62. Pritchard CC, Kroh E, Wood B, et al. Blood cell origin of circulating microRNAs: a cautionary note for cancer biomarker studies. Cancer Prev Res (Phila). 2012 Mar; 5(3):492–497. [PubMed: 22158052]
63. Levine AM, Karim R, Mack W, et al. Neutropenia in human immunodeficiency virus infection: data from the women's interagency HIV study. Arch Intern Med. 2006 Feb 27; 166(4):405–410. [PubMed: 16505259]
64. Zon LI, Arkin C, Groopman JE. Haematologic manifestations of the human immune deficiency virus (HIV). Br J Haematol. 1987 Jun; 66(2):251–256. [PubMed: 3606961]
65. Lagos-Quintana M, Rauhut R, Yalcin A, Meyer J, Lendeckel W, Tuschl T. Identification of tissue-specific microRNAs from mouse. Curr Biol. 2002 Apr 30; 12(9):735–739. [PubMed: 12007417]
66. Price JC, Thio CL. Liver disease in the HIV-infected individual. Clin Gastroenterol Hepatol. 2010 Dec; 8(12):1002–1012. [PubMed: 20851211]
67. Palella FJ Jr, Baker RK, Moorman AC, et al. Mortality in the highly active antiretroviral therapy era: changing causes of death and disease in the HIV outpatient study. J Acquir Immune Defic Syndr. 2006 Sep; 43(1):27–34. [PubMed: 16878047]
68. Xu J, Wu C, Che X, et al. Circulating microRNAs, miR-21, miR-122, and miR-223, in patients with hepatocellular carcinoma or chronic hepatitis. Mol Carcinog. 2011 Feb; 50(2):136–142. [PubMed: 21229610]
69. Xu J, Zhu X, Wu L, et al. MicroRNA-122 suppresses cell proliferation and induces cell apoptosis in hepatocellular carcinoma by directly targeting Wnt/beta-catenin pathway. Liver Int. 2012 May; 32(5):752–760. [PubMed: 22276989]
70. Qu KZ, Zhang K, Li H, Afdhal NH, Albitar M. Circulating microRNAs as biomarkers for hepatocellular carcinoma. J Clin Gastroenterol. 2011 Apr; 45(4):355–360. [PubMed: 21278583]
71. Trebicka J, Anadol E, Elfimova N, et al. Hepatic and serum levels of miR-122 after chronic HCV-induced fibrosis. J Hepatol. 2012 Oct 22; 8278(12):00814–00818.
72. Wang K, Zhang S, Marzolf B, et al. Circulating microRNAs, potential biomarkers for drug-induced liver injury. Proc Natl Acad Sci U S A. 2009 Mar 17; 106(11):4402–4407. [PubMed: 19246379]
73. Fu X, Han Y, Wu Y, et al. Prognostic role of microRNA-21 in various carcinomas: a systematic review and meta-analysis. Eur J Clin Invest. 2011; 41(11):1245–1253. [PubMed: 21521185]
74. Wang B, Zhang Q. The expression and clinical significance of circulating microRNA-21 in serum of five solid tumors. J Cancer Res Clin Oncol. 2012 Oct; 138(10):1659–1666. [PubMed: 22638884]
75. Engels EA, Biggar RJ, Hall HI, et al. Cancer risk in people infected with human immunodeficiency virus in the United States. Int J Cancer. 2008 Jul 1; 123(1):187–194. [PubMed: 18435450]
76. Ferracin M, Zagatti B, Rizzotto L, et al. MicroRNAs involvement in fludarabine refractory chronic lymphocytic leukemia. Mol Cancer. 2010; 9(123):123. [PubMed: 20504344]

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

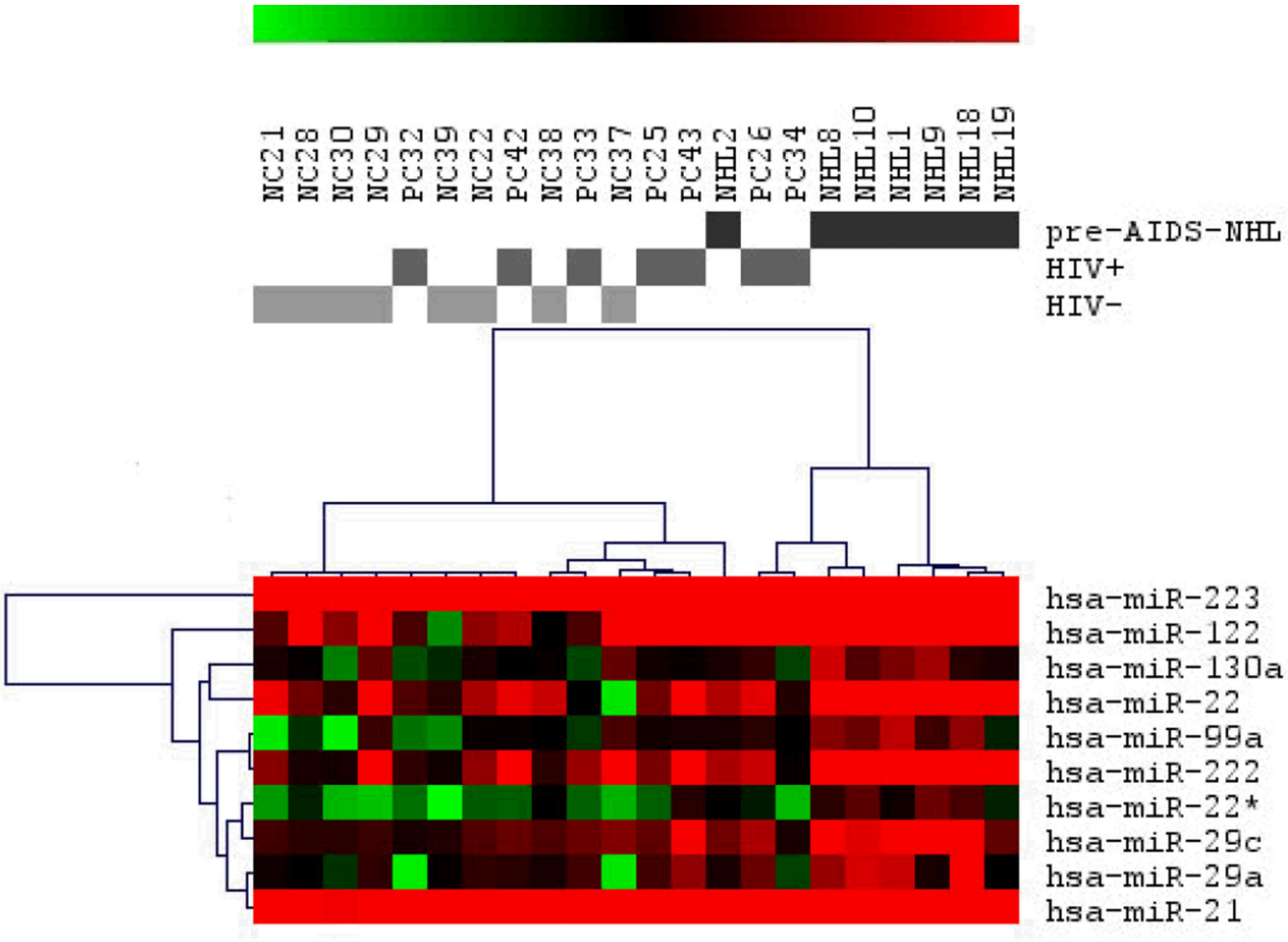

Fig. 1. Differentially expressed miRNAs identified during screening

Heatmap of miRNAs identified as differentially expressed between HIV−, HIV+, and pre-AIDS-NHL groups by significance analysis of microarrays test (false discovery rate=0%). Relative expression levels are depicted in red (high) and green (low).

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

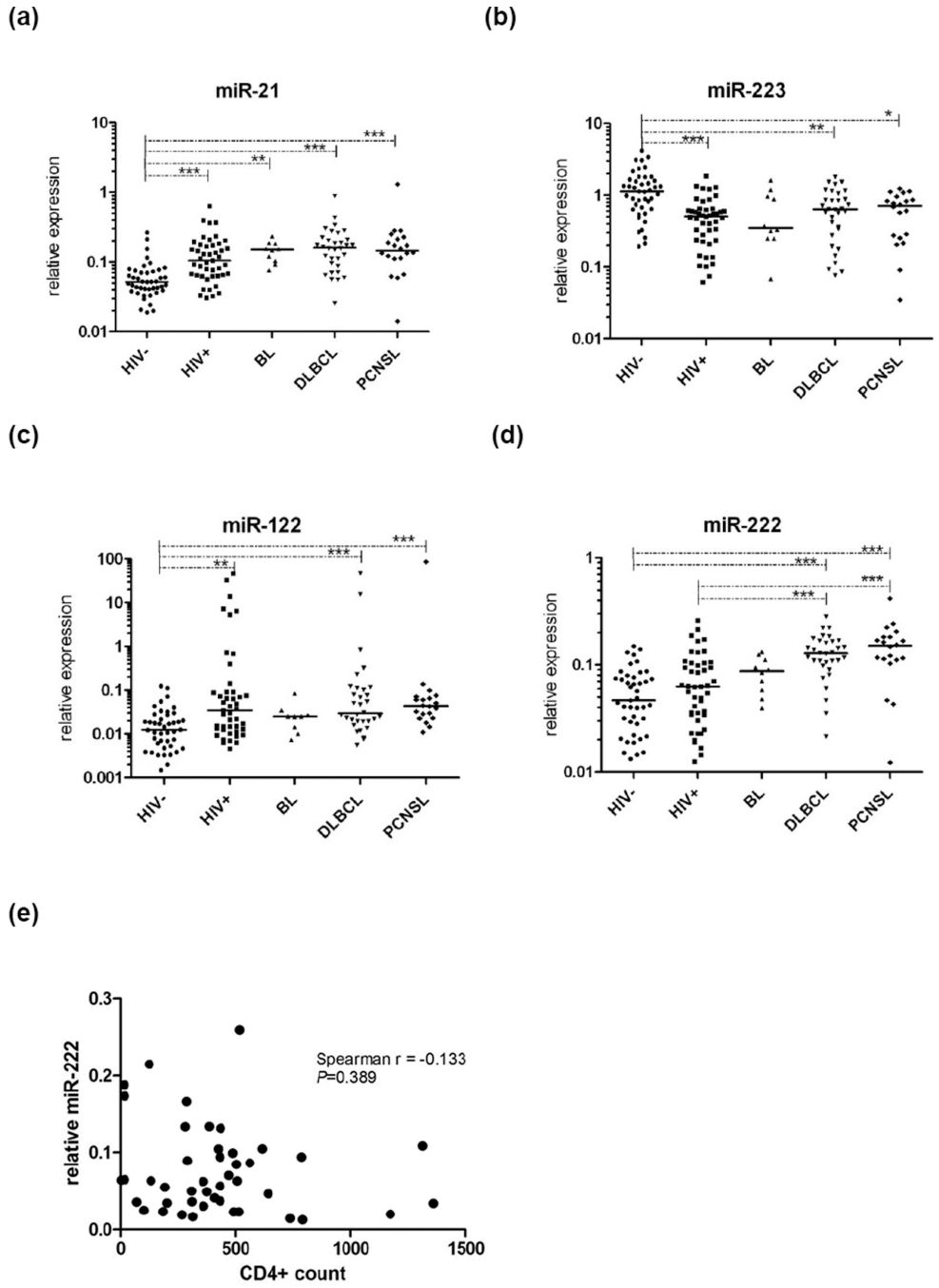

Fig. 2. Validation of serum miRNA levels by qPCR

Levels of (a) miR-21, (b) miR-223, (c) miR-122, and (d) miR-222 were assessed by qPCR in HIV−, HIV+, and pre-AIDS-NHL subjects. Comparisons that yielded significant differences are identified by asterisk(s) (* $p<0.05$; ** $p<0.01$; *** $p<0.001$). miRNA levels were normalized to levels of miR-16. Horizontal lines represent median values. *p* values were from Kruskal-Wallis test with Dunn's multiple comparisons. (e) Spearman's correlation analysis of miR-222 levels with CD4+ counts in the HIV+ group. BL, Burkitt

lymphoma; DLBCL, diffuse large B-cell lymphoma; PCNSL, primary central nervous system lymphoma.

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

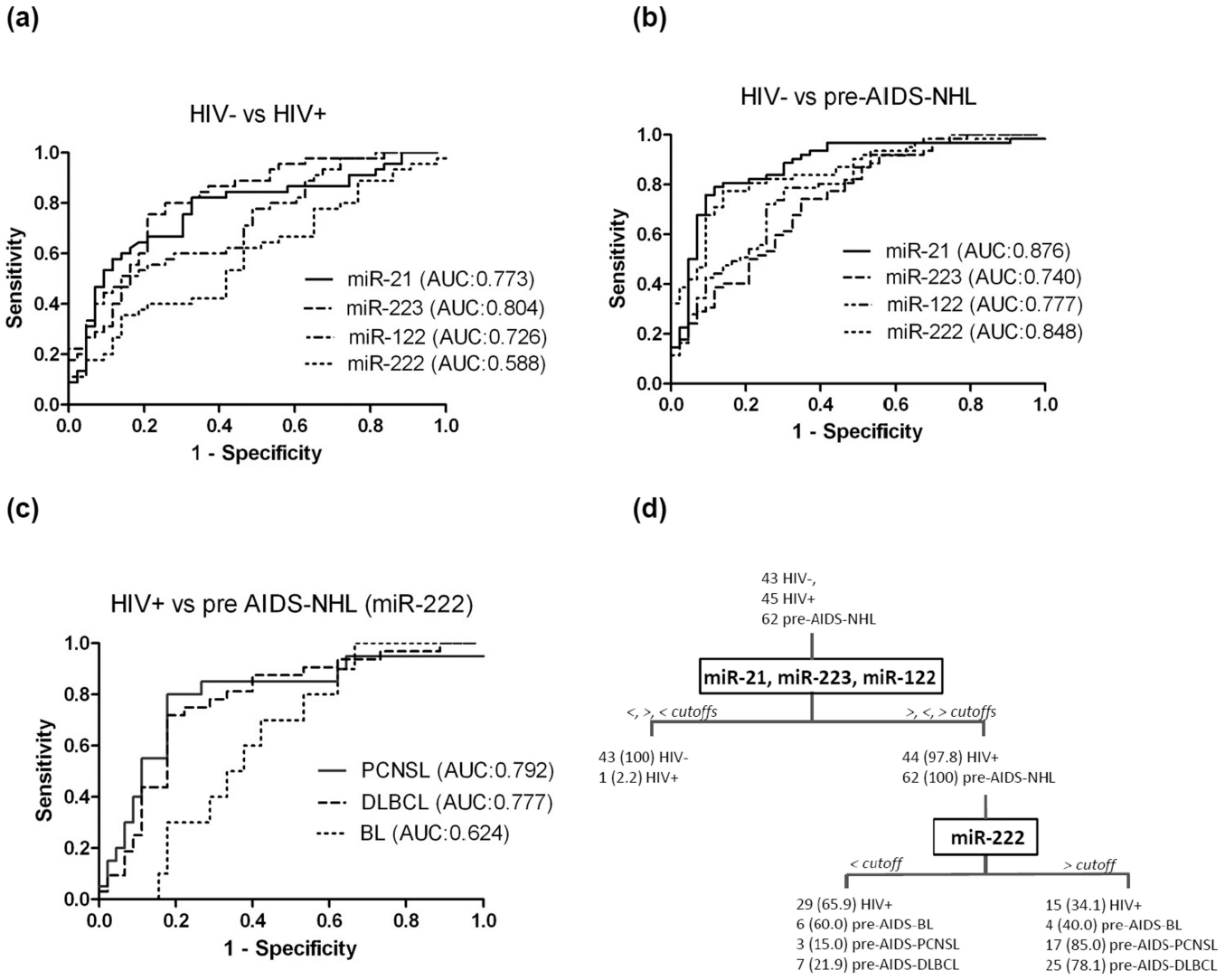

Fig. 3. Receiver-operating characteristics curve analysis

(a) HIV− group vs HIV+ group. The area under curve, AUC (95% confidence interval, CI) was 0.773 (0.673–0.873) for miR-21, 0.804 (0.712–0.897) for miR-223, 0.726 (0.662–0.830) for miR-122, and 0.588 (0.468–0.707) for miR-222. $p<0.001$ for all except miR-222 ($p=0.154$). (b) HIV− group vs pre-AIDS-NHL group. AUC (95% CI) was 0.876 (.0804–0.948) for miR-21, 0.740 (0.643–0.836) for miR-223, 0.777 (0.686–0.868) for miR-122, and 0.848 (0.773–0.922) for miR-222. $p<0.001$ for all. (c) HIV+ group vs pre-AIDS-NHL subgroups. AUC (95% CI) was 0.777 (0.670–0.884) for DLBCL, 0.792 (0.663–0.920) for PCNSL, and 0.624 (0.469–0.779) for BL. $p<0.001$ for DLBCL and PCNSL, $p=0.221$ for BL. (d) miRNA based classification of the 150 serum samples. Cutoff values were 0.094 (miR-21), 0.920 (miR-223), 0.024 (miR-122), and 0.091 (miR-222) and were determined based on the average of the median (relative expression levels) of the groups being compared. Numbers in parenthesis denote percentages. BL, Burkitt lymphoma; DLBCL, diffuse large B-cell lymphoma; PCNSL, primary central nervous system lymphoma.

NIH-PA Author Manuscript
NIH-PA Author Manuscript
NIH-PA Author Manuscript

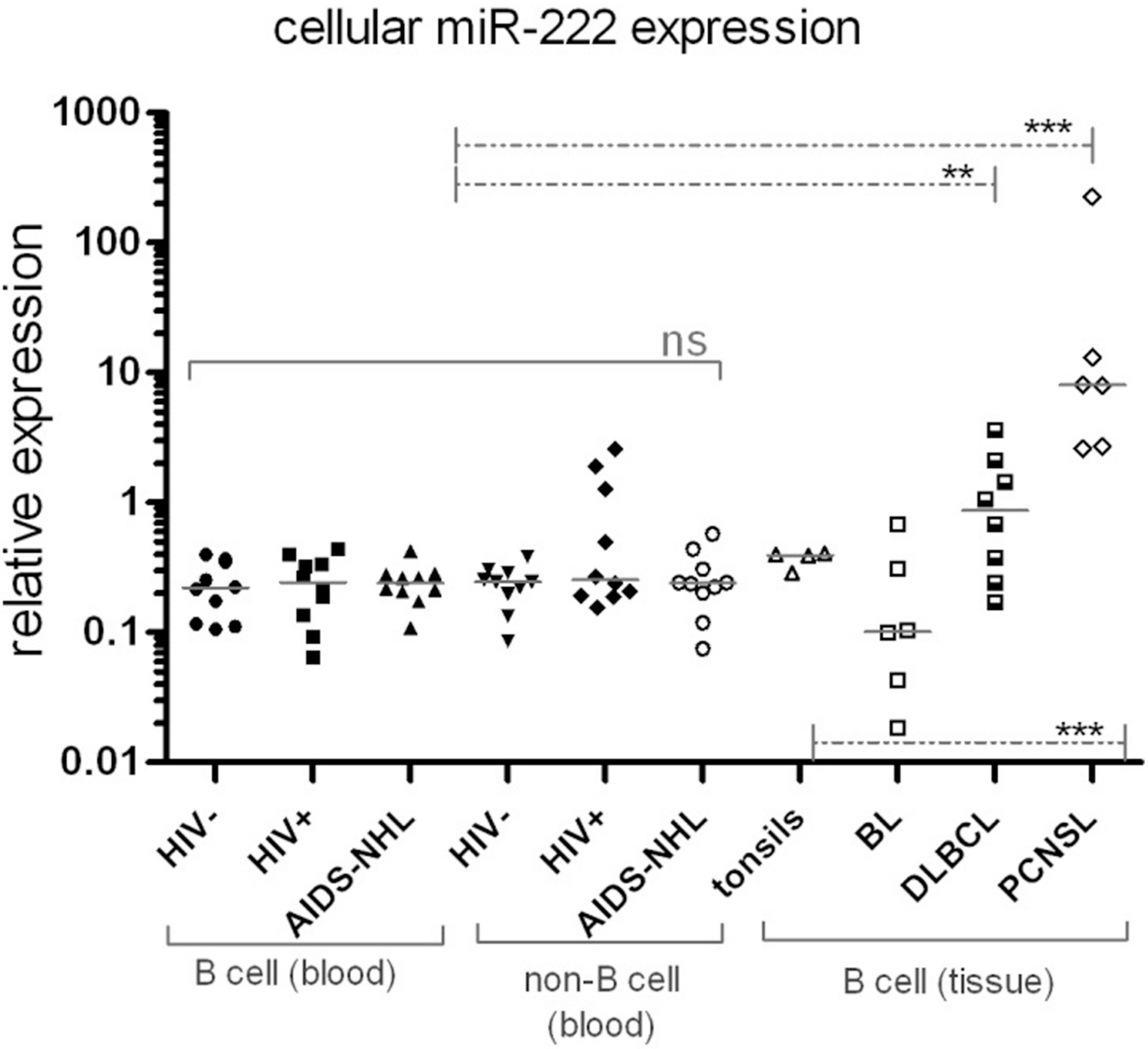

Fig. 4. miR-222 is overexpressed in DLBCL and PCNSL tumors

Relative level of miR-222 was assessed in cells from blood (B and non-B cell fraction from PBMC from the three subject groups) and tissues (tonsils and primary AIDS-related BL, DLBCL, and PCNSL tumors). miR-222 expression was normalized to small nucleolar RNU 48. Significant differences by Dunnett's multiple comparison (to blood cells) are identified by asterisk(s) (* $p<0.05$; ** $p<0.01$; *** $p<0.001$). BL, Burkitt lymphoma; DLBCL, diffuse large B-cell lymphoma; PCNSL, primary central nervous system lymphoma.

NIH-PA Author Manuscript
NIH-PA Author Manuscript
NIH-PA Author Manuscript

Table 1

Select characteristics of HIV negative (HIV−), HIV positive without NHL (HIV+), and HIV positive with subsequent NHL diagnosis (pre-AIDS-NHL) subject groups. All subjects are gay/bisexual men from the MACS.

		age in years	**CD4 count**	**HIV positivity duration in years**	**months preceding NHL diagnosis**
	N	**median (range)**	**avg ± SD**	**avg ± SD**	**median (range)**
HIV− (HIV negative)	43	43 (24–70)	995 ± 314	n/a	n/a
HIV+ (HIV positive without NHL)	45	36 (27–58)	424 ± 308	5.2 ± 3.0	n/a
(HIV positive with subsequent NHL)					
Burkitt lymphoma (BL)	*10*	*39 (25–44)*	*316 ± 205*	*4.4 ± 2.4*	*7.2 (3.1–20.6)*
Diffuse large B-cell lymphoma (DLBCL)	*32*	*41 (28–58)*	*160 ± 139*	*5.9 ± 3.5*	*8.4 (2.5–21.5)*
primary CNS lymphoma (PCNSL)	*20*	*40 (24–55)*	*82 ± 153*	*5.5 ± 3.5*	*10.3 (3.2–19.3)*
pre-AIDS-NHL	62	40 (24–58)	160 ± 172	5.5 ± 3.3	8.8 (2.5–21.5)