THESIS DOCTORAL

A frailty index predicts survival and incident multimorbidity independent of markers of HIV disease severity

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Contents

1. **Changes in clinical spectrum of HIV**
    a. Life expectancy in the immune-recovery era: the evolving scenario of the HIV epidemic in Northern Italy

2. **Drug toxicities**
    a. Morphological and Metabolic Components of Lipodystrophy in Various Nevirapine- Based Highly Active Antiretroviral Therapy (HAART) Regimens
    b. Switching to darunavir/ritonavir monotherapy vs. triple-therapy on body fat redistribution and bone mass in HIV-infected adults: Monarch randomized controlled trial.

3. **Comorbidities**
    a. Metabolic
        i. Combine use of waist and hip circumference to identify abdominally obese HIV-infected patients at increased health risk.
        ii. CD8 T-cell activation is associated with lipodystrophy and visceral fat accumulation in antiretroviral therapy-treated virologically suppressed HIV-infected patients.
        iii. Metabolic Alterations in HIV-Infected Pregnant Women: Moving to Metabolic Tailoring of Antiretroviral Drugs.
    b. Neoplastic
        i. Non-melanoma skin cancers among HIV-infected persons in the HAART-era
        ii. Sorafenib for the treatment of unresectable hepatocellular carcinoma in HIV-positive patients.
    c. Bone
        i. BMD changes across menopause in HIV infected women

4. Metodology

a. A frailty index predicts survival and incident multimorbidity independent of markers of HIV disease severity

5. Ultrasound

a. Evaluation with contrast ultrasound of the prevalence of splenic infarction in left-sided infective endocarditis
b. Contrast-enhanced ultrasound (CEUS) appearance of hepatic myelolipoma

List of original publications

Guaraldi G, Cossarizza A, Franceschi C, Roverato A, Vaccher E, Tambussi G, **Garlassi E**, Menozzi M, Mussini C, D'arminio Monforte A.

Life expectancy in the immune recovery era: the evolving scenario of the HIV epidemic in northern Italy.

J Acquir Immune Defic Syndr. 2014 Feb 1;65(2):175-81. doi: 10.1097/QAI.0000000000000018.

Guaraldi G, Zona S, Orlando G, Carli F, Stentarelli C, Luzi K, **Garlassi E**, Menozzi M, Bagni P, Adorni F.

Morphological and metabolic components of lipodystrophy in various nevirapine-based highly active antiretroviral therapy (HAART) regimens: a cross-sectional, observational study.

Clin Drug Investig. 2011 Nov 1;31(11):759-67. doi: 10.2165/11593920-000000000-00000.

Guaraldi G, Zona S, Cossarizza A, Vernacotola L, Carli F, Lattanzi A, Nardini G, Orlando G, **Garlassi E**, Termini R, Garau M.

Switching to darunavir/ritonavir monotherapy vs. triple-therapy on body fat redistribution and bone mass in HIV-infected adults: Monarch randomized controlled trial.

Int J STD AIDS. 2014 Mar;25(3):207-12. doi: 10.1177/0956462413497701. Epub 2013 Aug 28.

O'Neill T, Guaraldi G, Orlando G, Carli F**, Garlassi E**, Zona S, Després JP, Ross R.

Combine use of waist and hip circumference to identify abdominally obese HIV-infected patients at increased health risk.

PLoS One. 2013 May 20;8(5):e62538. doi: 10.1371/journal.pone.0062538. Print 2013.

Guaraldi G, Luzi K, Bellistrì GM, Zona S, Domingues da Silva AR, Bai F, **Garlassi E**, Marchetti G, Capeau J, Monforte Ad.
CD8 T-cell activation is associated with lipodystrophy and visceral fat accumulation in antiretroviral therapy-treated virologically suppressed HIV-infected patients.
J Acquir Immune Defic Syndr. 2013 Dec 1;64(4):360-6.

Guaraldi G, Stentarelli C, Da Silva AD, Luzi K, Neri I, Cellini M, Petrella E, **Garlassi E**, Menozzi M, Facchinetti F, Mussini C.
Metabolic alterations in HIV-infected pregnant women: moving to metabolic tailoring of antiretroviral drugs.
AIDS Rev. 2014 Jan-Mar;16(1):14-22.

Garlassi E, Harding V, Weir J, Francis N, Nelson M, Tom Newsom-Davis T. & Bower M.
Non-melanoma skin cancers among HIV-infected persons in the HAART-era.
J Acquir Immune Defic Syndr. 2012 Jun 1;60(2):e63-5.

Berretta M, Di Benedetto F, Dal Maso L, Cacopardo B, Nasti G, Facchini G, Bearz A, Spina M, **Garlassi E**, De Re V, Fiorica F, Lleshi A, Tirelli U.
Sorafenib for the treatment of unresectable hepatocellular carcinoma in HIV-positive patients.
Anticancer Drugs. 2013 Feb;24(2):212-8.

Garlassi E, Menozzi M, Stentarelli C, Zona S, Domingues da Silva A Rochira V, Santoro A, Carli F, Brigante G, Beghetto B, Nardini G′ Mussini C, Vescini F, Yin M, Guaraldi G.
BMD changes across menopause in HIV infected women.
SUBMITTED.

Guaraldi G, Brothers TD, Zona S, Stentarelli C, **Garlassi E**, Carli F,

Malagoli F, Santoro A, Menozzi M, Mussi C, Mussini C, Kirkland S, Falutz J, Rockwood K.

A frailty index predicts survival and incident multimorbidity independent of markers of HIV disease severity.

SUBMITTED to Lancet Infect Dis.

Menozzi G, Maccabruni V, Gabbi E, Magnani G, **Garlassi E.**

Evaluation with contrast ultrasound of the prevalence of splenic infarction in left-sided infective endocarditis.

J of Ultrasound. 2014. doi 10.1007/s40477-014-0131-4.

Menozzi G, Maccabruni V, Marini G, Froio E, **Garlassi E.**

Contrast-enhanced ultrasound (CEUS) appearance of hepatic myelolipoma.

J Ultrasound. 2014. doi 10.1007/s40477-014-0137-y.

From 2007 to 2013 I worked in Infectious Disease Clinic striving to improve the quality of life for people living with HIV through the delivery of comprehensive primary medical care.

Since I was a medical student I've always been fascinated in both Infectious Diseases and Onco-hematology. During my residency in Infectious Diseases I managed to establish scientific collaboration with Aviano National Cancer Institute, focusing my attention on clinical management of HIV-associated cancers.
In 2011 I moved to London as a clinical observer at Chelsea and Westminister Hospital (Kobler Day Care), the leading UK centre for the treatment of HIV-related cancers.
I have broad interests in the field of HIV/AIDS: epidemiology and prevention of HIV infection, including behavioral and biomedical prevention, treatment and adverse effects of antiretroviral therapy as HIV lipodystrophy and metabolic toxicities.
Up to date my research interests have focused on lipodystrophy and metabolic toxicities of antiretroviral therapy, aging and frailty in HIV and on malignancies associated with HIV infection and other infections (hepatitis).

As Research Fellow in Metabolic Clinic of Infectious Disease Department (Modena) I had the opportunity to be involved in the management and treatment of a great number of patients with HIV infection.

I worked with a very broad and engaged network of research collaborators in Hematology and Oncology Department. I think that the interaction between behavioral, pshicological, pharmacological and clinical aspects both related to HIV and oncologic condition is a challenge for researchers.

I attended International Doctorate School in Clinical and Experimental Medicine because the educational program was a great opportunity for a young doctor to acquire skills required in clinical research at Universities and Hospitals.

International Doctorate School made me better understand how to apply basic research to clinical practice and helped me in planning my original research.

Through my PhD work and ongoing research, I focused my attention on some of the emerging problems in HIV population (aging, non-infectious comorbidities, pregnancy, frailty, HAART toxicities, non-melanoma skin cancers etc), collaborating with international experts.

Objectives

Primary objective

The common main objectives of the studies herein presented are:

- to elucidate the changes in clinical spectrum of HIV infection during the last decade
- to investigate the clinical impact of comorbidities and aging in HIV patients

Secondary objectives

1. Epidemiological

To estimate LE in people living with HIV receiving HAART compared to that of the general population and to assess the impact of immune-recovery

2. Clinical

a. to investigate the effect of therapy on morphological and metabolic components of lipodystrophy in HIV-infected patients;

b. to study the prevalence and risk factors for several common age-related noninfectious comorbidities (NICM) among HIV-infected persons
c. to evaluate frailty and metabolic alterations in HIV patients
d. To evaluate the impact of skin and liver cancer in HIV patients
e. to apply the use of ultrasound to the every-day clinical practice

1. Changes in clinical spectrum of HIV

Highly active antiretroviral therapy (HAART) for HIV infection has been a terrific medical success story: clinical AIDS is no longer the inevitable outcome of HIV infection in countries with good access to treatment and a disease that was previously associated with extremely high mortality rates is now generally thought of as a chronic condition.

This evolving scenario signalled a profound change in the epidemiological approach to HIV epidemic. The classification of HIV history in Pre- and Post-HAART eras, initially introduced to describe the reduction in mortality observed in antiretroviral treated patients, nowadays depicts an increase of life expectancy (LE) in people living with HIV.

In recent years, both national cohorts, and inter-cohort studies have described the improvements in life expectancy of HIV positive individuals. However, given the diverse ages at which LE is reported, and the different populations under study (which have variable LEs), it is extremely difficult to compare the results from these studies.

Estimates of LE among adults with HIV infection are not usually quoted from birth, but from an exact age (often 20 or 25 years) in specific sub-populations, such as people experiencing seroconversion, or at the time of AIDS diagnosis, entry into care or HAART initiation. Recently, the Collaboration of Observational HIV Epidemiological Research in Europe (COHERE) group identified the circumstances under which treated HIV-infected individuals would be expected to experience similar mortality rates to those of the general population. Over 80,000 HAART-experienced individuals were included in the study. Death rates among men, but not women, reached those of the matched general population when individuals had attained (and maintained) a CD4 count >500 cells/mm^3 on HAART for at least three years.

Despite the differences between the studies, it is noteworthy that all

studies that have described LE in this population have noted the strong association between increased LE and the immune-recovery induced by HAART.

Immunological improvement has not been defined consistently in the HIV literature, but it is usually dependent on two measurements of the CD4 cell count: the individual's lowest CD4 count measured prior to starting HAART (the 'nadir' CD4 count, a reflection of where the patient has come from) and the individual's latest CD4 count on HAART (reflecting the person's current status); the former is often categorised as above or below 350 cells/mm^3, whereas the latter is more commonly categorised as above or below 500 cells/mm^3.

a. Life expectancy in the immune-recovery era: the evolving scenario of the HIV epidemic in Northern Italy

AIM

Our retrospective observational study aimed to estimate LE in people living with HIV receiving HAART compared to that of the general population living in the same country, i.e. northern Italy, and to assess the impact of immune-recovery, defined as a current CD4 count >500 cells/mm^3 following HAART initiation among individuals with a CD4 count nadir prior to HAART of <350 cells/mm^3 on LE.

Conclusions

This is the first paper to report LE in HAART-treated individuals with HIV in Italy and a comparison of LE with that seen in the general northern Italian population.

The improvement in LE may be explained by several factors: an upward trend in LE in the general population, changing demographics and risk

factors of the HIV population, a decline in the transmission of drug-resistance virus strains, access to modern HAART regimens a good immune response of patients on HAART. These factors are, however, unlikely to contribute to improvements in LE homogeneously in different epidemiological settings.

Notably no difference in proportion of patients experiencing immunerecovery was shown in the pre and post-HAART groups suggesting that this immunological phenomenon is driven by a complex interplay of drugs, virus and host factors.

We bring forwards this idea concentrating in the immune-recovery phenomenon. We focused our analyses on a population of HAART treated patients who had or had not experienced immune-recovery on treatment. Whilst or categorization of immune-recovery is arbitrary, it comprises two immunological determinants: a measure of the persons most advanced immunodeficiency, and their current status. In future years, this categorization may become obsolete, if treatment guidelines move towards recommending initiation of ART at higher CD4 counts, or even to all HIV infected individuals regardless of their CD4 count. However, under current clinical practice, this categorization remains clinically relevant given the high number of HIV-infected people who are unaware of their HIV status and still represents the majority of individuals who will experience immune-recovery following initiation of HAART.

The strength of our study is the description of immune-recovery as a phenomenon strictly related to calendar year which characterized the changing clinical picture of HIV disease. Analogue to the recent definition of “Community Viral load” we can define “HAART community immune-recovery” the overall rate of patients undergoing HAART attaining immune-recovery. HAART community immune-recovery was appreciable few years after HAART introduction and apparently showed an increase in 2006. From this date, immune-recovery prevalence was associated with a

significant reduction of AIDS related death. We therefore suggest that this shifting pattern of HIV disease is better described by the HAART community immune-recovery appreciable from 2006 rather than the start of HAART era in 1996.

In conclusion this study shows that within the HIV infected population the proportion of subjects that has achieved a satisfactory immune-recovery has increased over time. As a consequence, a higher proportion of patients approaches LE of the general population.

2. Drug toxicities

Three main changes in body composition have been reported during clinical trials of antiretroviral treatment: lipoatrophy, lipohypertophy and reductions in bone mineral density. These changes can contribute to patient quality of life, stigma and morbidity. Morphological abnormalities (lipoatrophy and central fat accumulation) and metabolic changes (dyslipidaemia and glucose regulation impairment) have emerged as components of lipodystrophy and as major tolerability issues with long-term use of highly active antiretroviral therapy (HAART) in HIV-positive patients. Protease inhibitors (PIs) are recognized as having the greatest impact in terms of metabolic complications, followed by nucleoside reverse transcriptase inhibitors, while the non-nucleoside reverse transcriptase inhibitors (NNRTIs) have the least impact.

Different antiretroviral treatments can have a range of conflicting effects on these three measures of body composition.
Lipoatrophy is the loss of sub-cutaneous fat from the limbs, buttocks and face. In previous studies, lipoatrophy has been most correlated with use of thymidine analogues – zidovudine or stavudine. Switching from stavudine or zidovudine to either tenofovir or abacavir has led to improvements in lipoatrophy. However, several studies have shown no difference in the risk of lipoatrophy between abacavir or tenofovir),

despite differences in the lipid profiles of these nucleoside analogues. Several randomised studies have also shown no difference in the risk of lipoatrophy between protease inhibitors, non-nucleosides or integrase inhibitors, when used in combination with nucleoside analogues. However, analyses from three randomized trials, using DEXA scanning, have shown increases in limb fat after stopping all nucleoside analogues and switching to protease inhibitor monotherapy, compared to the continued use of triple combination treatment including nucleoside analogues.

Lipohypertophy is the accumulation of fat in the abdominal cavity (Visceral Adipose Tissue (VAT)), dorso-cervical fat pad, breasts or other lipomas. Lipohypertrophy has been associated mainly with the use of protease inhibitors, with few or no significant differences seen between them. Lipohypertrophy, especially increases in Visceral Adipose Tissue and waist circumference, could increase cardiovascular risk and overall mortality.

Osteopenia is the loss of bone mineral density, either from the spine, legs or total bone mass. In naïve patients, loss of bone mineral density was also associated with use of tenofovir, especially when combined with atazanavir/ritonavir.

a. Morphological and Metabolic Components of Lipodystrophy in Various Nevirapine- Based Highly Active Antiretroviral Therapy (HAART) Regimens

Regimens based on the NNRTI nevirapine have been shown to achieve significant metabolic benefits and may help to improve dyslipidaemia. Improvements in body shape changes associated with lipodystrophy have also been reported when nevirapine replaced a PI in long-term triple therapy.

AIM

The objective of this cross-sectional observational ('real-world') study was to investigate the effect of three HAART regimens plus stable nevirapine therapy on morphological and metabolic components of lipodystrophy in HIV-infected patients.

Conclusions

Our results demonstrate that stable nevirapine, combined with background NRTIs with a metabolic risk similar to that of NNRTIs, has very little impact on lipodystrophy in patients with HIV and lipodystrophy. This regimen therefore represents a suitable treatment option for these patients. Further study is warranted to establish if nevirapine overcomes the distinct lipodystrophy risk profile associated with different nucleoside backbone regimens.

b. Switching to Darunavir/ritonavir monotherapy vs. triple-therapy on Body Fat Redistribution and Bone Mass in HIV-Infected Adults: MONARCH randomized controlled trial

PI/r monotherapy is increasingly used as a novel antiretroviral strategy in the new paradigm of induction-maintenance and appear promising in the setting of an aging HIV infected patients vulnerable to cumulative exposure of NRTI.

Previous studies have shown that a switch to tenofovir, or *de novo* use of tenofovir, leads to reductions in bone mineral density compared with a control nucleoside analogue, typically abacavir.

AIM

The primary objective of the study is to show the impact of switching to Darunavir/ritonavir monotherapy vs. triple-therapy on Body Fat Redistribution and Bone Mass in the two comparative groups.

Conclusions

These data provide clinical interpretation of the metabolic changes previously shown in the MONET and MONOI studies.

We described a significant rise in bone mineral density after discontinuation of tenofovir+emtricitabin.

Clinical trials of protease inhibitor monotherapy allow for analysis of the effects of stopping nucleoside analogues completely. Most analyses of body composition changes have been conducted either to compare different nucleoside analogues, or to compare different third antiretrovirals among groups of patients who are all taking two nucleoside analogues.

If improvements in bone mineral density after stopping tenofovir could be reproduced in other studies, this may be an important treatment option for ageing patients with low bone mineral density.

There was a significant reduction in HOMA insulin resistance during the trial, across the two treatment arms.

Patients in the DRV/r monotherapy arm showed significantly larger rises in limb fat than those switched to DRV/r plus two nucleoside analogues. In the Monarch trial there was a trend for rises in trunk fat, measured by DEXA, in both treatment arms. Even so, the levels of visceral adipose tissue remained stable over time in the two treatment arms. Further long-term analyses of body composition in larger trials will be required, to identify the clinical consequences of these changes.

3. Comorbidities

The widespread introduction of highly effective combination antiretroviral therapy (ART) for human immunodeficiency virus (HIV) infection has dramatically decreased HIV-associated morbidity and mortality. Despite marked increases in life expectancy, mortality rates among HIV-infected persons remain 3– 15 times higher than those seen in the general

population. Although some of the excess mortality observed among HIV-infected persons can be directly attributed to illnesses that occur as a consequence of immunodeficiency, more than half of the deaths observed in recent years among ART-experienced HIV-infected
patients are attributable to noninfectious comorbidities (NICMs). These include cardiovascular disease (CVD), hypertension (Htn), bone fractures, renal failure, and diabetes mellitus (DM), diseases that in the general population often coexist and are associated with advancing age.

Specific age-related NICMs and polipathology are more common among HIV-infected patients than in the general population. The prevalence of Pp in HIV-infected persons anticipated Pp prevalence observed in the general population among persons who were 10 years older, and HIV-specific cofactors (lower nadir CD4 cell count and more prolonged ART exposure) were identified as risk factors. These data support the need for an aggressive approach to the screening, diagnosis, and treatment of NICMs as part of routine healthcare for HIV-infected patients.
It has been hypothesized that the increased prevalence of NICM observed among HIV-infected patients, compared with the general population, is the result of premature aging in the former.
Additional studies are needed to further evaluate the impact of convergent age-related NICMs on age-related functional status, frailty, and disability among ART-experienced HIV-infected persons and to provide insights into accelerated aging processes that may be associated with chronic HIV infection.

a. Metabolic

HIV-infected patients receiving antiretroviral treatment frequently accumulate fat at the abdominal level.

i. **Combine use of waist and hip circumference to identify abdominally obese HIV-infected patients at increased health risk.**

Given that lower body subcutaneous adipose tissue is a common site for loss of adipose tissue in HIV-infected adults, a strong argument exists for the routine acquisition of WC and HC in clinical settings. Studies in non-HIV individuals investigated the associations of WC and HC with morbidity, demonstrating a protective effect of an increased HC for a given WC and/or BMI.

AIM

To determine whether for a given waist circumference (WC), a larger hip circumference (HC) was associated with a reduced risk of insulin resistance, type 2 diabetes (T2D), hypertension and cardiovascular disease (CVD) in HIV-infected patients. A second objective was to determine whether, for a given WC, the addition of HC improved upon estimates of abdominal adiposity, in particular visceral adipose tissue (VAT), compared to those obtained by WC alone.

CONCLUSIONS

The primary finding of this study was that the identification of HIV-infected patients at increased risk by WC alone is substantially improved by the addition of HC. These observations reinforce the recommendation that WC and HC should be used in combination to identify higher-risk HIV-infected adults, in particular men, and that identification of HIV-infected adults with elevated health risk may be underestimated when WC is used alone. Our observations also suggest that the ability of WC and HC combined to predict VAT might not be the conduit by which these anthropometric measures combined identify those at increased risk of cardiometabolic risk and morbidity.

To our knowledge no prior study has determined whether HC adds to the

health risk identified by WC alone in HIV-infected patients.

Our findings that derive from the use of simple anthropometric tools that are readily available to the practitioner are consistent with studies in HIV-infected patients wherein sophisticated radiographic methods were used. Our findings demonstrate that the identification of HIV- infected individuals at increased health risk by WC alone is substantially improved by the addition of HC. It is possible that HC in combination with WC identifies ectopic fat deposition in tissues other than VAT including liver and/or epicardial tissues both of which are associated with increased health risk.

Further study with large sample sizes is required to develop valid algorithms or cutpoint values that could ultimately be incorporated into routine clinical practice to help identify high-risk HIV patients.

ii. CD8 T-cell activation is associated with lipodystrophy and visceral fat accumulation in antiretroviral therapy-treated virologically suppressed HIV-infected patients.

It is unknown whether T-cell activation and immune phenotypes are associated with fat accumulation.

AIM:

Search for an association between the presence of clinical lipodystrophy (LD), visceral and subcutaneous abdominal adipose tissue amount (VAT and SAT), and peripheral T-cell immune phenotypes.

CONCLUSIONS:

This study established the presence of a relationship between T-cell phenotypes and adiposity in ART-treated HIV-infected patients. We observed that both clinical lipodystrophy and visceral fat amounts were associated with peripheral CD8 T-cell activation suggesting that CD8 activation could be involved in central fat accumulation, in particular in VAT amount. We also confirmed the association between VAT and insulin

resistance as a predictor of type 2 diabetes and CAC as a cardiovascular risk factor.

In conclusion, our data provide further evidence that in HIV-infected patients ART-controlled, CD8+ T cell activation may reveal a potential link with lipodystrophy and VAT accumulation which represents a strong cardio-metabolic risk factor.

iii. Metabolic Alterations in HIV-Infected Pregnant Women: Moving to Metabolic Tailoring of Antiretroviral Drugs.

The most striking effect of increased survival and improved quality of life in HIV-infected women undergoing antiretroviral therapy is the feasibility of motherhood-desire satisfaction. However, such advantages are often associated with drug-related metabolic toxicities, particularly relevant in the pregnancy context. Recent guidelines provide recommendations and trends for the use of antiretroviral therapy in pregnant women, but current literature falls short of providing specific insights on the need for metabolic monitoring and treatment in HIV-infected pregnant women.

Pregnancy is a metabolic transition process, potentially associated with specific diseases in the mother, in the newborn, and in the adulthood of the child. Close monitoring is needed to diagnose metabolic alterations that can lead to adverse outcomes in the mother, in the newborn, and potentially in adulthood. Lifestyle interventions and an appropriate metabolic tailoring of antiretroviral therapy drugs need to be considered in the prevention and treatment of metabolic alteration during pregnancy.

In this review we provide specific insight into the state-of-the-art of: detection, evaluation, and management of metabolic alterations in this special population. We will not simply discuss antiretroviral therapy metabolic toxicities, but rather their interaction with the physiological metabolic changes occurring during pregnancy.

AIM

– To review metabolic changes during pregnancy and describe their impact both in the mother and the newborn

– To discuss how to monitor/deal with the clinical management of metabolic disorders

– To discuss the metabolic tailoring of ART in pregnant women

Conclusions

Pregnancy itself contributes to metabolic derangements during pregnancy; pregnancy is a transitory state in the metabolic syndrome.

Close monitoring is needed to diagnose metabolic alterations that can lead to adverse outcomes in the mother, in the newborn, and potentially in adulthood.

A metabolically tailored management of ART must be considered.

Drug safety (with particular regard to new ART) in pregnant women must account for metabolic abnormalities and its impact at organ level as well as for tocolytic activity and teratogenicity.

Lifestyle interventions and an appropriate metabolic tailoring of ART drugs need to be considered in the prevention and treatment of metabolic alteration during pregnancy.

b. Neoplastic

With the availability of highly active antiretroviral treatment (HAART) in 1996, a remarkable decrease in HIV-related morbidity and mortality was observed. This decline was associated with a relative significant increase in morbidity and mortality related to many different non–HIV-related causes, including cancers.

The increase in life expectancy achieved means that HIV-infected patients are now exposed both to the potential long-term effects of treatment and

virus itself and to age-related problems, but less is known about the contribution of each parameter on giving cancers in HIV positive people.
In the management of HIV patients, oncological co morbidities are an important issue since the fact that the prevalence of many type of cancers in HIV infected people is higher than in the general population and that the clinical behavior of some cancers seems to be more aggressive.
In addition, the associated reduction in AIDS-defining cancers such Kaposi's sarcoma and non- Hodgkin's lymphomas has been accompanied by an increase in non-AIDS-defining cancers.

i. Non-melanoma skin cancers among HIV-infected persons in the HAART-era

Since the advent of highly active antiretroviral therapy (HAART), there has been increasing attention on the non-AIDS defining cancers, including non-melanoma skin cancers (NMSC). Non-melanoma skin cancers (NMSC) are the most common malignancies in the UK with nearly 100,000 cases registered in 2008 although registration is known to be incomplete. Immunosuppression has been associated with an increased risk of NMSC particularly amongst recipients of solid organ transplants. Epidemiological surveys of the incidence of non AIDS defining malignancies in seropositive populations usually exclude NMSC, although recent studies have reported an increased relative risk of NMSC in people living with HIV. Immunosuppression, in particular following solid organ transplantation is associated with an increased risk of NMSC. Amongst transplant recipients, squamous cell cancers predominate and the risk appears to rise with increasing duration and doses of immunosuppression. Epidemiological analyses of the risk of NMSC in people living with HIV have produced conflicting results and a meta-analysis suggests that the standardised incidence ratio whilst elevated is considerably lower than that observed following solid organ transplantation.

AIM

Describe pathological details and outcomes of NMSC in HIV seropositive individuals.

Conclusions

The adverse histological characteristics in BCC were not associated with more advanced immunosuppression whether measured as CD4 cell count, plasma HIV viraemia, HAART use or duration, length of time HIV infected or nadir CD4 cell count. Similarly, the histological grade of the SCC tumours was not correlated with any of these surrogate markers of immune function.

In summary, non-melanoma skin cancers are part of the growing list of cancers that may be encountered in patients living longer with chronic HIV-infection. Although our patients presented at a younger age and frequently with more aggressive pathological subtypes, non-melanoma skin cancers do not appear to be significantly associated with immune function or HAART.

ii. Sorafenib for the treatment of unresectable hepatocellular carcinoma in HIV-positive patients.

Hepatocellular carcinoma (HCC) is the third most common cause of cancer-related death and the advanced stage has a very poor prognosis, with a median survival time of 6–9 months. The coinfection with HIV results in greater viral replication and accelerates the clinical course of the disease. HCC, secondary to viral hepatitis, has emerged as a major source of morbidity and mortality in HIV-infected patients; indeed, the risk of HCC is seven-fold higher in HIV-infected patients than in uninfected patients. Moreover, the risk for HCC in HIV-coinfected and hepatitis virus-coinfected patients is unlikely to be modified with the use of HAART.

Therapeutic options for HCC are stage dependent: however, as cancer-

related symptoms often emerge after a long-standing asymptomatic period, HCC is often diagnosed at an advanced stage.

Until recently, no effective treatment was available for patients with HCC not amenable to resection or transplantation, moreover, to date, there are few data available on treatment for HCC in HIV-infected patients. Sorafenib, an oral multikinase inhibitor, blocks tumor cell proliferation and exerts an antiangiogenic effect. Sorafenib has shown a good tolerability in patients with HCC, making sorafenib the standard of care for the treatment of patients with unresectable HCC. Thus, little is known about the safety and efficacy of sorafenib and concomitant HAART in HIV-infected patients with unresectable HCC.

Furthermore, although HAART agents are well known to cause drug–drug interactions, little is known about a possible drug–drug interaction between HAART and sorafenib.

AIM

Describe the safety and efficacy of sorafenib in HIV-infected patients with unresectable hepatocellular carcinoma (HIV-u-HCC) and concomitant highly active antiretroviral therapy (HAART).

Conclusions

The median time to progression and overall survival was 5.1 (range 0.5–13.3) and 12.8 (range 1.1–23.5) months, respectively. Grades 3–4 toxicities included diarrhea (four patients, 14.8%), hypertension (three patients, 11%), and hand-and-foot skin reaction (four patients, 14.9%). Most drug-related side effects were low grade and manageable. This retrospective study shows favorable survival data among HIV-u-HCC patients treated with sorafenib together with a reasonable safety

On the basis of our results, the administration of sorafenib-based regimens and concomitant HAART may be reasonably safe and effective in

patients with advanced HCC and HIV infection, who generally have a poor prognosis. The preliminary results appear promising, either providing clinically relevant information or aiding therapeutic decision-making for improved outcomes in this setting of patients.

c. Bone

HIV infection has been associated with low bone mineral density (BMD) and high fracture risk in numerous cross sectional and longitudinal studies.

Meta-analysis of longitudinal studies showed a short-term accelerated BMD loss in cohorts initiating HAART but stabile BMD in cohorts on established HAART**.** Risk factors for BMD change are multifactorial including classical risk factors (family history for hip fracture, low body weight, vitamin D deficiency, steroid use and gonadal status) as well as HAART exposure, with tenofovir being the agent most consistently associated with bone loss, and HIV per se.

i. BMD changes across menopause in HIV infected women

HIV-infected postmenopausal women have higher rates of bone loss than HIV negative women.

Rates of change in bone mineral density (BMD) have been studied in pre and in post menopausal HIV-infected women but never across the menopausal transition.

AIM

Examine rates and predictors of bone loss across menopause in HIV women.

Conclusions

This is the first study analyzing BMD across menopause. Our study demonstrated that BMD was stable in the pre-menopause period, and bone loss was more rapid in the early than late menopausal period, and associated predominantly with traditional risk factors.
Current TDF exposure was independently associated with BMD lowering in early menopause, but not in the late menopausal stage or in the model that included all menopausal stages. The clinical significance of the impact of TDF use during the menopausal transition remains uncertain.

At present the majority of HIV infected women in industrialized countries are entering the menopause transition period. In this scenario, properly designed clinical studies are needed to address immediately effective interventions to reduce the burden of osteoporosis and fracture risk in the post-menopausal period.

4. Metodology study

a. A frailty index predicts survival and incident multimorbidity independent of markers of HIV disease severity

Aging with HIV increases risk for many age-related health problems – the so-called HIV-associated non-AIDS (HANA) conditions, including cardiovascular disease, osteoporosis, chronic obstructive pulmonary disease, and non-AIDS cancers – which span multiple physiologic systems and causes.[1] People who develop one of these conditions are more likely to accumulate further health problems. Multimorbidity contributes to further vulnerability and complexity in clinical management and justifies intensifying chronic disease prevention strategies in the contemporary antiretroviral therapy era.[2]

Interest is strong in methods to identify individuals at risk of multimorbidity and other adverse health outcomes in addition to

mortality. In geriatric medicine such multisystem vulnerability is quantified using the construct of frailty, which is a state of increased vulnerability related to the degradation of homeostatic mechanisms.[3] Although often linked to old age, frailty can be described across the life course[4] including among individuals with acquired[5] vulnerability states.

Two different conceptual models inform frailty measurement. One describes frailty as a clinical phenotype comprised of at least three of five specific, age-associated health deficits: shrinking (malnutrition or sarcopenia), diminished strength, reduced exercise tolerance, slowness, and low activity levels.[6] Another model describes frailty as the cumulative effects of general health deficits that are more likely to be acquired with age.[7] The proportion of such deficits present from a group of at least 30 health variables, known as a frailty index (FI), indicates frailty severity. The FI approach enables grading across a continuum of vulnerability, from fittest to frailest, allows frailty to be estimated from diverse health variables, and can more sensitively grade risk of adverse outcomes.[8,9] Although employed successfully in other areas,[3,7,10] the utility of an FI in identifying vulnerability among people aging with HIV is unknown.

In the present study, we sought to construct an FI from health variables collected as part of routine assessments in an HIV clinic. We assessed the validity of the FI, described the characteristics of frailty in one clinical cohort, and evaluated the ability of an FI to predict mortality and incident multimorbidity.

Methods

Setting & sample

This is a secondary analysis of data from the prospective Modena HIV Metabolic Clinic (MHMC) cohort. The multidisciplinary metabolic clinic at the University of Modena and Reggio Emilia School of Medicine in Modena,

Italy, was initiated in 2004 to comprehensively assess longitudinal metabolic changes among people with HIV.[1]

Frailty index (FI)

An FI calculates the proportion of health deficits individuals have accumulated out of a group of relatively non-specific health variables. Health variables, including signs and symptoms of disease, laboratory measures, and self-reported data, can be included in an FI if they meet some basic criteria: they should characterize acquired health deficits that are generally age-related, and as a group, must cover a range of physiologic systems.[7] While certain health deficits might have small effects on their own, their cumulative effects can be large. If at least ~30 variables are included, the number of deficits appears to be much more informative than the specific nature of the deficits.[7,11]

Health measurements collected as part of regular clinic assessments were screened for inclusion in the frailty index. We selected health variables that were not representative of HIV disease severity or immune deficiency. As we intended to assess the ability of a frailty index to predict incident multimorbidity as well as survival, we also excluded from the FI variables used to define our multimorbidity outcome (described below).

Each variable included in the FI was recorded with values of one when a deficit was present and zero when absent.[7] Missing values were removed from both numerator and denominator of the FI, and observations were included if they had at least 80% of available health variables at that visit.[9]

Validation strategy (content: cosa misura e mancanza di patient related outcome, come si confronta con cquello che hai) NON DEVE CONTENERE ETA'

We evaluated the validity of the FI using a three-part approach, assessing content, construct, and criterion validity.[12] Content validity is assayed by whether the approach being used is sensible to experts in the field (or "sensible on its face"). In this case, included health variables were all among those routinely collected through assessments at an HIV clinic. To assess construct validity, we compared characteristics of this FI to those consistently demonstrated by FIs in HIV-negative cohorts.[7,11] These include the relationship between FI score and age (on a log scale, around 1% in clinical or institutional samples); distribution of FI scores (usually Gaussian in clinical samples); and the existence of a submaximal limit to frailty, e.g. 99% of people have FIs less than 1 (and usually less than 0.7).[7] Among people with FI scores closer to this upper limit, the relationship between frailty and increasing age attenuates and approaches zero, as people die rather than accumulate more deficits.[13] We also evaluated associations between FI scores and other measures of HIV-related vulnerability: current and nadir CD4 cell count, HIV viral load, and Veterans Aging Cohort Study (VACS) Index score.[14] To test criterion validity, we evaluated the ability of the FI to independently predict two clinically meaningful outcomes in this setting: mortality and incident multimorbidity.

Outcomes

Vital status is regularly updated in MHMC via telephone contact and hospital records. We defined multimorbidity as the presence of two or more of the following eight health problems: cardiovascular disease (clinical diagnosis); hypertension (blood pressure measured twice >135/85 or on treatment); type two diabetes mellitus (measured glucose ≥126 or oral glucose tolerance test >200 or on treatment); chronic kidney disease (estimated glomerular filtration rate <60 mL/min); cirrhosis (FIB-4 >3.25); chronic obstructive pulmonary disease (FEV1/FVC ratio <70); osteoporosis (dual-energy x-ray absorptiometry T- or Z-score <-2.5 or fragility fracture); or cancer (clinical diagnosis). We included

multimorbidity as an outcome as it has been identified as a meaningful health state among people living with HIV,[1] and because mortality in this cohort was expected to be relatively low.[15]

Covariates

Age was recorded at each study visit, and gender at baseline. Current and nadir CD4 cell counts and HIV viral load measurements were treated as continuous variables for correlation analyses, and when included in regression models they were grouped into clinically relevant categories. CD4 cell count was divided into four groups (<100, 101-350, 351-500, and >500 cells/mm^3). Viral load was categorized as detectable or undetectable (≤40 copies/mL). Smoking history was assessed at each study visit; pack-years were calculated and grouped into 10-year intervals. Injection drug use was assessed at baseline.

Analysis cox:

Descriptive statistics and FI scores at first visit were calculated and distributions visualized. Cross-sectional relationships between log-FI scores and years of age were evaluated by linear regression.[7,11] To identify potential attenuation in the relationship of frailty and increasing age among frailer participants, we also performed regressions on age among participants with FI scores in the top 50^{th}, 85^{th}, and 95^{th} percentiles. Pearson correlation coefficients assessed cross-sectional associations between FI and other continuous health measures. For prediction models, FI scores were categorized into 0.1 increments. Each covariate was first evaluated in univariate prediction models, and covariates significantly associated with the outcome were then combined in multivariable models. Cox regression models were used to assess survival, and hazard ratios (HRs) with 95% confidence intervals (CIs) were calculated. Incident multimorbidity prediction analysis included participants without multimorbidity at baseline. We used longitudinal generalized estimating equation models for panel data and calculated

incident rate ratios (IRRs) with 95% CIs. Censoring occurred at the study visit when incident multimorbidity was first diagnosed, or at most recent visit.

In further exploratory analyses, we modified the original FI to create two additional FIs: one with the 8 comorbidity variables added, and another with comorbidity and HIV severity and immune deficiency markers also added. We then compared the ability of these of FIs to classify risk for 2- and 5-year mortality using receiver operator characteristic (ROC) curves, by calculating the area under the curve (AUC) with 95% CIs. Statistical significance was set a $p<0.05$, and data were analyzed using SPSS 21.0.0 (IBM Corp., Armonk, NY, USA).

Ethics

Approval for the Modena HIV Metabolic Clinic Cohort was obtained from the Research Ethics Board of the University of Modena and Reggio Emilia, and all participants provided written consent at their initial clinic visit.

Results

Following established criteria, 37 variables were selected for inclusion in an FI (Table 1). 2,722 participants in the MHMC cohort provided 9,784 study visits with available FI scores. Participants were generally middle aged, and most were male with undetectable viral load and CD4 counts above 500 cells/mm^3 (median 557, IQR 400-726 cells/mm^3; Table 2).

FI scores at first visit ranged from 0·00 to 0·63 (mean 0·31±0.10; median 0·30) and were normally distributed (Figure 1). FI scores increased exponentially with age (Figure 2). On log scale, FI scores increased by 1·8% (2/3 of one deficit) with each year of age ($p<0·001$). FI scores increased 0·4% with each year of age ($p<0·001$) among patients with FI at or above the sample mean; 0·1% among participants with FI scores in the top 85th percentile (≥0·42, $p=0·1$), and 0·1% among

participants in the 95th percentile (≥0·48, 0·1%, p=0·2). FI scores at first visit were modestly correlated with nadir CD4 (r=-0·15, p<0.001), and VACS Index (r=0·34, p<0·001), but not with current CD4 (r=-0·04, p=0·07) or viral load (r=-0·02, p=0·5).

There were 34 deaths in the sample over 8206 person-years follow-up (PYFU; 0·41/100 PYFU). In univariate analyses, significant predictors of survival were FI (HR 2·32, 95% CI 1·53-3·52), age (1·05, 1·00-1·09), current CD4 (0·37, 0·24-0·54), nadir CD4 (0·42, 0·24-0·75) and injection drug use (3·56, 1·81-7·01). When these were combined in a multivariate model, FI, current CD4, and injection drug use remained significant (Figure 3).

88% (n=2,383) of participants did not have multimorbidity at baseline. Of these, 232 (9·7%) acquired multimorbidity during follow-up. In univariate analysis, FI score (IRR 2·34, 1·98-2·76), age (1·09, 1·07-1·11), female gender (0·41, 0·27-0·61), current CD4 (0·65, 0·54-0·78), pack-years smoking (1·20, 1·09-1·31), and IDU (1·47, 1·07-2·02) were each predictive of incident multimorbidity. When these factors were combined in multivariate analysis, FI score, age, gender, and current CD4 count remained significant (Figure 4).

We then compared the ability of three FIs to classify mortality risk: the 37-item FI, an FI with the 8 co-morbidity variables added (45 items total), and an FI with both these and 8 HIV-related variables added (53 items total). Area under the receiver operating characteristic curve increased with more items in the FI, but these differences were not significant (Table 3).

Discussion

In a large, well-characterized cohort of HIV-positive men and women in northern Italy, we assessed frailty by constructing an FI from routinely

collected health variables that were not measures of immune deficiency or HIV disease severity. We found that the FI exhibited expected characteristics and was able to predict survival and multimorbidity independent of age, behavioral, and HIV-related variables. We further found that FIs with added variables, including HIV-related variables, discriminated mortality risk similarly to the FIs without these variables.

Our results should be interpreted with caution. There were relatively few endpoints during follow-up, which could have affected our estimates from regression models. However this potential lack of statistical power increased our risk of Type II error, and so did not influence the direction of our findings that frailty was a significant predictor of both survival and multimorbidity. Parameter estimates were also generally stable between univariate and multivariate analyses. While further follow-up is needed to capture more data related to mortality, the low mortality rate reflects increased life expectancy with HIV.[15] As such, it is important to evaluate relationships with other clinically important outcomes related to aging, including geriatric syndromes, mobility limitations, and cognitive decline.[16,17] Further, while the FI used here incorporated health measures across a range of physiologic systems, it did not include deficits in clinical domains normally included in geriatric assessments and often included in frailty scales such as cognition, function, or mood.[9] The FI comprised health variables regularly assessed in an HIV clinic, rather than geriatric assessment. While evidence of the validity of the FI as constructed here is encouraging, the FI approach should also be cross-validated in other HIV-positive cohorts using different variables in different settings. These questions are motivating active inquiry by our group.

Panel. Research in context.

Systematic review

A recent review summarized what is known about frailty in people aging with HIV.[18] We supplemented this review by searching Cochrane Library, CINAHL, Embase, Google Scholar, PubMed, and PyschINFO using the terms "frail" or "frailty" along with "human immunodeficiency virus" or "HIV." Additional papers were identified from reference lists of retrieved articles, Google Scholar linking of articles citing retrieved articles, and personal libraries of the authors.

Interpretation

Previous studies of frailty in people with HIV have applied phenotypic frailty scales, based on the assessment of a few, specific criteria. This is the first study to explore frailty in relation to deficit accumulation among people aging with HIV. Our findings using this approach support those of previous studies, including the associations of frailty with age, and with current CD4 count.[19–25] One study identified associations between frailty and higher HIV viral load,[21] which we did not find here, but our cohort had much higher rates of viral suppression (93%). A recent study identified that phenotypic frailty independently predicted hospitalization and mortality in the Veterans Aging Cohort Study (VACS) cohort.[26] Otherwise few longitudinal outcomes have been assessed in relation to frailty among people aging with HIV, especially among clinical cohorts.[18] The present study identifies dose-response relationships of frailty with risk for mortality and for multimorbidity independent of HIV-related and behavioral factors. The predictive abilities of frailty assessment and changes in frailty over time should be explored further in HIV-related settings.

Applying the FI approach enabled us to synthesize a large amount of routinely collected clinical information to assess health and frailty. Many scales measure frailty, and just what scale is best among people with HIV has not been established. Previous reports of frailty in people with HIV have assessed phenotypic frailty,[18] and the VACS index has also been

proposed as a measure of frailty in people aging with HIV.[14] The VACS index incorporates health variables across multiple, but specific, physiologic systems, has been associated with health outcomes, including mortality, fragility fractures and neurocognitive impairment,[14,27] and was correlated with the FI in our study. However, the VACS index was developed specifically as a parsimonious prognostic tool, rather than to measure or characterize frailty. As such its algorithm includes chronologic age. In this way the VACS index differs from most frailty scales, which seek to quantify frailty independently from chronologic age.[3] The MHMC cohort does not currently capture the data necessary to calculate a frailty phenotype score, and VACS index scores were not available for all included participants. The infeasibility of calculating frailty phenotype and VACS index scores using the data routinely collected in the MHMC cohort illustrates that such rules-based approaches to defining frailty might be too specific, and not be appropriate in each individual case. Many experts will not doubt that health deficits are important, but some will be concerned by interactions between the items, and wish for a list of fewer variables to be used. The inclusion of more items enables more pieces of information to contribute to the overall understanding of the level of frailty in an individual.[7,28] The accumulation of many small pieces of information is also well accepted in disciplines such as biophysics, decision science, and computer science.[29]

The FI in this study exhibited characteristics consistently demonstrated by FIs and other frailty scales in HIV-negative populations.[7,11] FI scores had a normal distribution with a high mean score and a small, positive, nonlinear association with age that attenuated among the frailest participants (from 2% in the whole sample to zero in the frailest). These are all characteristic of frailty consistently observed among diverse clinical samples in HIV-negative populations.[5,7,11] Even though the mean FI score was high in this sample, the upper limit to frailty was not higher than

expected (maximum around 0.7), which suggests that the index itself was not overestimating scores.

We found that frailty and current CD4 count were each independently predictive of both survival and multimorbidity, and the addition of HIV-related variables into the FI did not significantly improve its ability to discriminate mortality risk. This suggests that frailty and current CD4 count might be considered independent signals in assessing or quantifying vulnerability among people aging with HIV.[18] While low CD4 count is an important clinical sign, an increasing proportion of HIV-positive individuals on treatment are achieving immune reconstitution, and indeed most participants in this clinical cohort had CD4 cell counts greater than 500 cells/mm^3 and undetectable viral load. Further, IDU was a predictor of survival, suggesting social vulnerability might play a role. The observational nature of this study did not allow us to demonstrate pathogenic mechanisms linking HIV disease severity, social vulnerability, frailty, and health outcomes. Understanding these links, including the development of frailty among people aging with high CD4 cell counts, independent from immune deficiency, is motivating further work.

The practical utility of regular frailty assessment in HIV care has yet to be explored.[18] The ability to measure frailty holds potential value in terms of both policy and clinical care.[3] Identifying frailty and understanding how health status is likely to change can assist planning for services that might be required, and clinical frailty assessment can help to weigh the risks and benefits of interventions, or predict risk of adverse outcomes.[3] Emerging information continues to suggest that frailty itself is treatable, and intervention is possible in preventing or delaying worsening frailty and functional decline.[3] Therefore, the frailty index could be investigated as a clinical tool, and as an endpoint in intervention trials among people aging with HIV. Further, the frailty index offers some means of quantifying the whole health of the individuals, so that the impact of a

large number of health deficits can be quantified parsimoniously, as a single variable. In this way, a key lesson of ageing – ("The problem of old age come as a package") can be addressed – one of the lessons that, as a recent Nature commentary put it, have "have hardly made a dent in human medicine".[28]

A major motivation to evaluate frailty and deficit accumulation in relation to aging with HIV came from clinical experience at the multidisciplinary MHMC, where patients are followed over time for a wide variety of health measures. Often patients improve in some measures and deteriorate in others, and it is difficult to assess whether a patient's health is improving or deteriorating overall. Based on what is known about frailty in HIV-negative populations, it was hoped that an FI based on cumulative health deficits might be able to provide an informative summary state variable to contribute to the holistic clinical evaluation of vulnerability.[5,7,30] In light of this, an algorithm has been developed within the MHMC electronic medical record that enables automatic and instant assessment of FI score with each update of a patient's chart. The FI is being used by clinicians to supplement clinical judgment about patient's health trajectories, vulnerability, and health surveillance.

Table 1. Health variables included in the frailty indices and description of deficit scoring

No.	Variable	Deficit description
	37-item frailty index	
1	Lipoatrophy	• Multicenter AIDS Cohort Study (MACS) criteria definition
2	Lipohypertrophy	• MACS criteria definition
3	Non-alcoholic fatty	• Liver/spleen ratio < 1·1

	liver disease	
4	Menopause or male hypogonadism	• If female: FSH>30 IU/L & LH<30 IU/L and/or absence of menstruation >1 year • If male: testosterone<300 ng/dL
5	High or low body mass index	• <18 or >25
6	High waist circumference	• If female: >88 cm • If male: >102 cm
7	High visceral adipose tissue	• VAT>130 cm2 or VAT/TATratio >0·5
8	Sarcopenia or presarcopenia	• Fat-free mass index < -1 SD
9	Insulin resistance	• HOMA-IR > 2·8
10	High total cholesterol	• > 200 mg/dL
11	High low density lipoprotein	• >100 mg/dL
12	Low high density lipoprotein	• < 40 mg/dL
13	High triglycerides	• >150 mg/dL
14	High homocysteine	• If female, > 10 µmol/L. If male, > 15 µmol/L.
15	Abnormal white blood cell counts	• < 4000 cells/µL
16	Anemia	• If female, < 10 grams/dL • If male, < 12 grams/dL.
17	Hepatitis C co-infection	• Positive
18	Hepatitis B co-infection	• Hepatitis B Antigen positive
19	Vitamin D insufficiency	• < 30 ng/mL

20	Polypharmacy	• > 5 drug classes (excluding antiretroviral therapy)
21	Abnormal parathyroid hormone	• > 60 pg/mL
22	Elevated D-dimer	• > sample mean (358)
23	Elevated C-reactive protein	• > 0.7 mg/L
24	Sedentary lifestyle	• < 3 hours/week physical activity
25	Atherosclerosis	• Coronary artery calcium score > 100 or intima media thickness > 0.85 mm
26	Hyponatremia	• < 125 mmol/L
27	Proteinuria or albuminuria	• > 5 mgm/mmol
28	Elevated aspartate transaminase (AST)	• > 31 U/L
29	Elevated alanine transaminase (ALT)	• > 31 U/L
30	Abnormal alkaline phosphatase	• < 38 or > 126 U/L
31	Elevated gamma-glutamyl transphosphatase (GGT)	• > 55 U/L
32	Low platelets	• < 150 billion/L
33	Abnormal potassium	• < 3·5 or > 5·3 mEq/L
34	Abnormal phosphorus	• < 2·5 or > 5·1 mg/dL
35	Abnormal thyroid stimulating hormone	• < 0·27 or > 4·2 mIU/L
36	Elevated total bilirubin	• > 1·10 mg/dL
37	Unemployment	• Self-report

Co-morbidities		
1	Cardiovascular disease	• Clinical diagnosis
2	Hypertension	• Measured blood pressure or on treatment
3	Diabetes mellitus type II	• Fasting glucose > 125 md/dL or on treatment
4	Chronic kidney disease	• estimated glomerular filtration rate < 60 mL/min/1·73m^2
5	Cirrhosis	• FIB-4 > 3·25
6	Chronic obstructive pulmonary disease	• Spirometry: FEV1/FVC ratio < 70
7	Osteoporosis	• Dual-energy x-ray absorptiometry (DEXA) T- or Z-score <-2·5 or fragility fracture
8	Cancer	• Clinical diagnosis
HIV-related variables		
1	Current CD4 cell count	• <500 cells/mm^3
2	Nadir CD4 cell count	• <200 cells/mm^3
3	Viral load	• >40 copies/mL
4	CD4/CD8 cell ratio	• <1·0
5	Duration of HIV infection	• >10 years
6	Pre-HAART start	• Start of antiretroviral therapy before 1/1/1997
7	History of AIDS	• History of CDC class C
8	ART failure	• History of viral load > 1000 copes/mL while on antiretroviral therapy

Table 2. Descriptive characteristics of the sample at first study visit.

Sample size, n	2,722
Age, mean ± SD	46 ± 8
Women, n (%)	867 (32)
Current CD4 cell count, mean ± SD	588 ± 267
Nadir CD4 cell count, mean ± SD	209 ± 163
Undetectable viral load, n (%)	2577 (93)
VACS Index, mean ± SD	16 ± 15
Pack-years smoking, mean ± SD	16 ± 16
Injection drug use, n (%)	730 (27)
Multimorbidity[1], n (%)	390 (14)
Cardiovascular disease, n (%)	95 (4)
Hypertension, n (%)	752 (28)
Type 2 Diabetes Mellitus, n (%)	254 (9)
Chronic Kidney Disease, n (%)	14 (1)
Hepatic cirrhosis, n (%)	150 (6)
Chronic obstructive pulmonary disease, n (%)	60 (2)
Osteoporosis, n (%)	368 (14)
Cancer, n (%)	26 (1)

[1]Multimorbidity was defined as having 2 or more comorbidities out of: hypertension, diabetes, chronic kidney disease, chronic obstructive pulmonary disease, osteoporosis, cancer.

Table 3. Area under receiver operating characteristic curve for prediction of 2-year and 5-year mortality with three different frailty indices.

Frailty index	**Description**	**AUC 2-year mortality (95% CI)**	**AUC 5-year mortality (95% CI)**
37-item frailty index	-	0·73 (0·62-0·87)	0·73 (0.65-0·81)
45-item frailty index	37-item frailty index + 8 comorbidity variables	0·91 (0·86-0·96)	0·80 (0·71-0·88)
53-item frailty index	37-item frailty index + 8 comorbidity variables + 8 HIV-related variables	0·88 (0·82-0·94)	0·83 (0·77-0·90)

AUC: Area under receiver operating characteristic curve. CI: confidence interval.

FIGURES

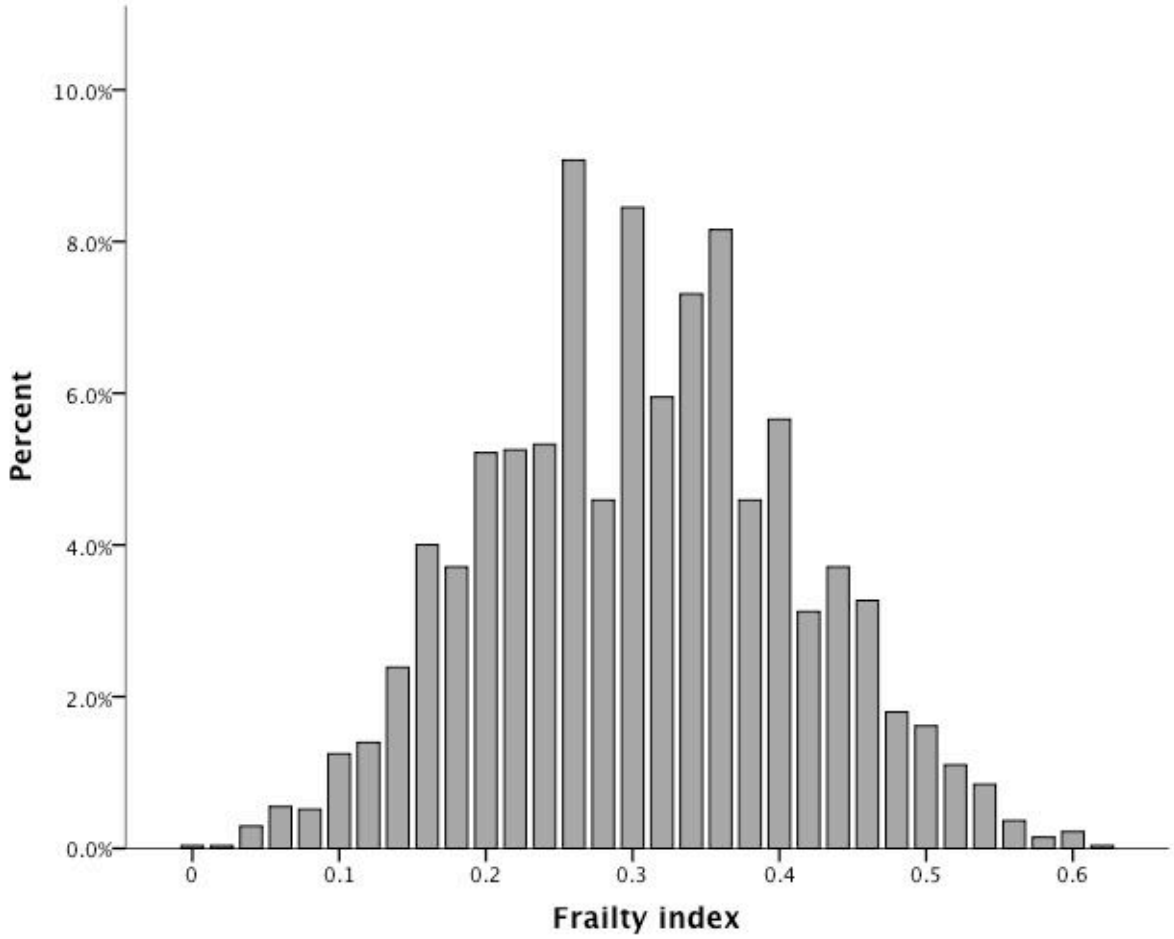

Figure 1. Distribution of frailty index scores at first visit.

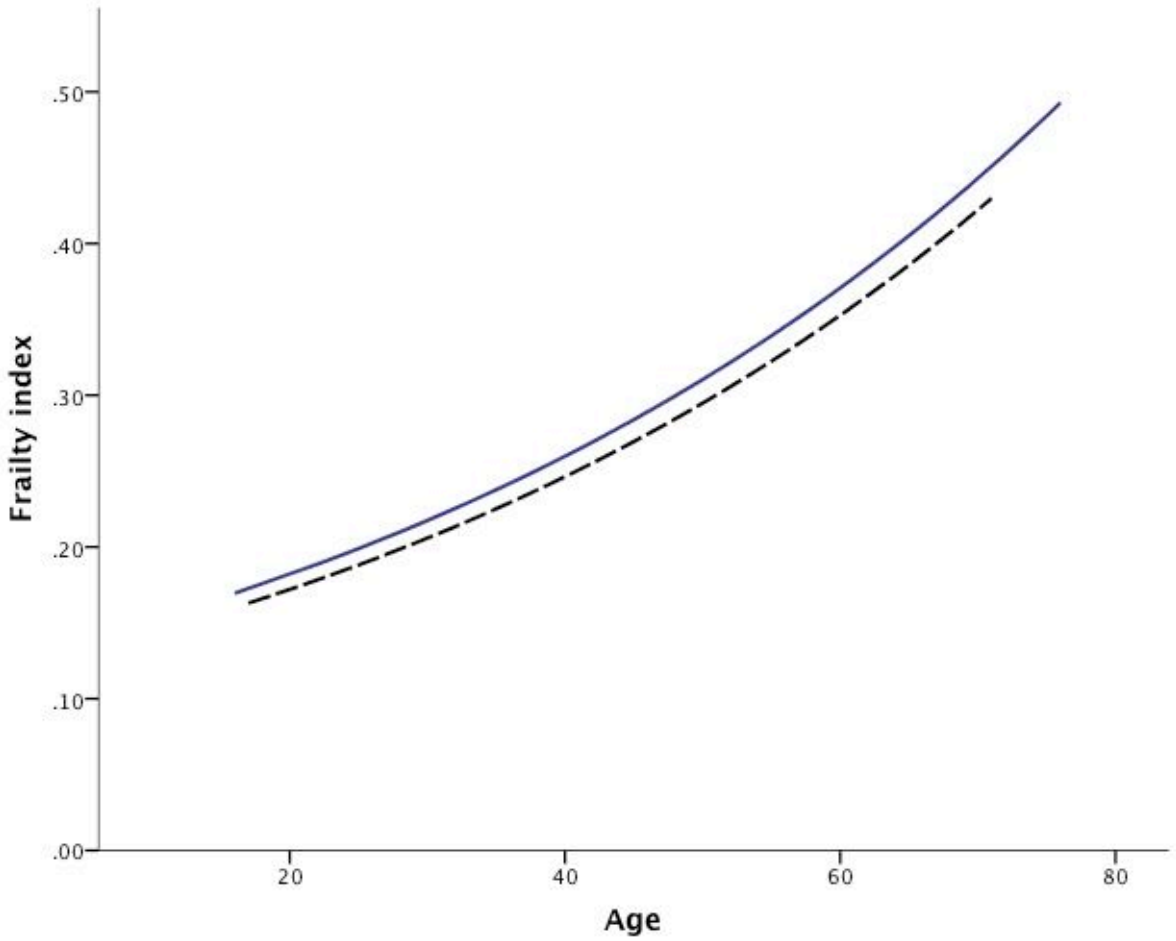

Figure 2. Average frailty index score at each age. Lines represent exponential best fit. Solid line is men, dashed line is women.

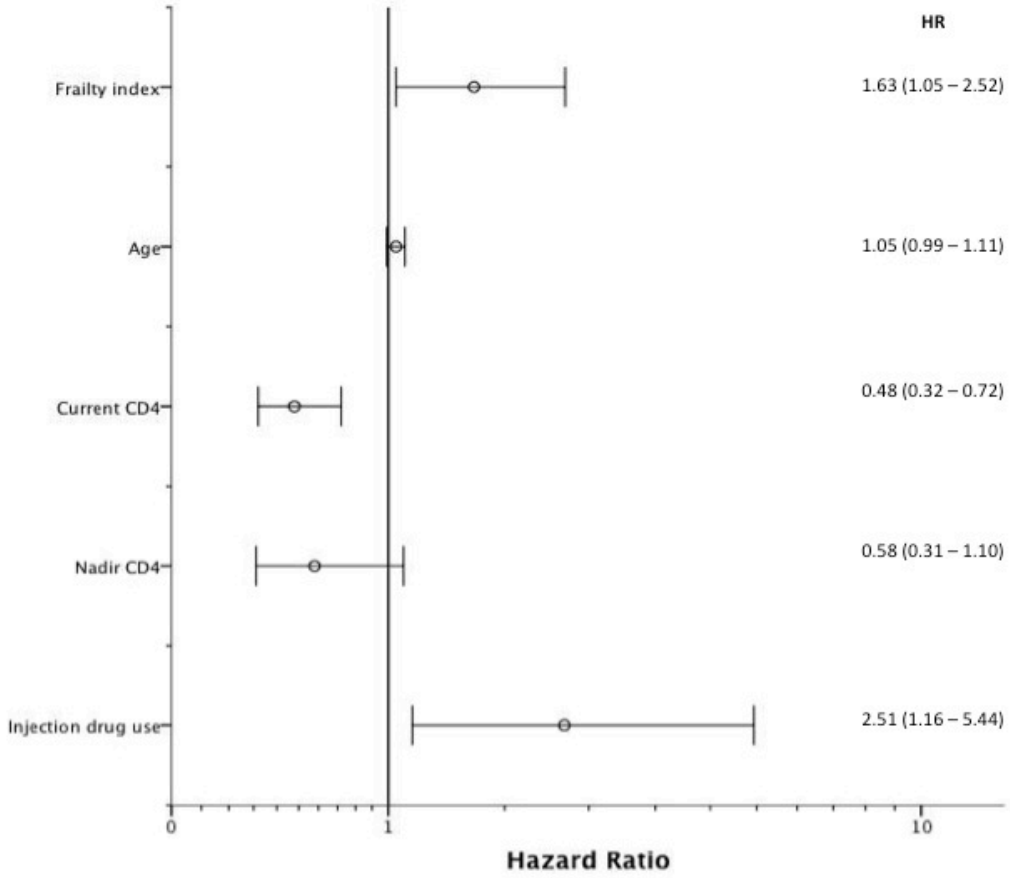

Figure 3. Predictors of survival in multivariate analysis. Points represent adjusted hazard ratios, and whiskers 95% confidence intervals.

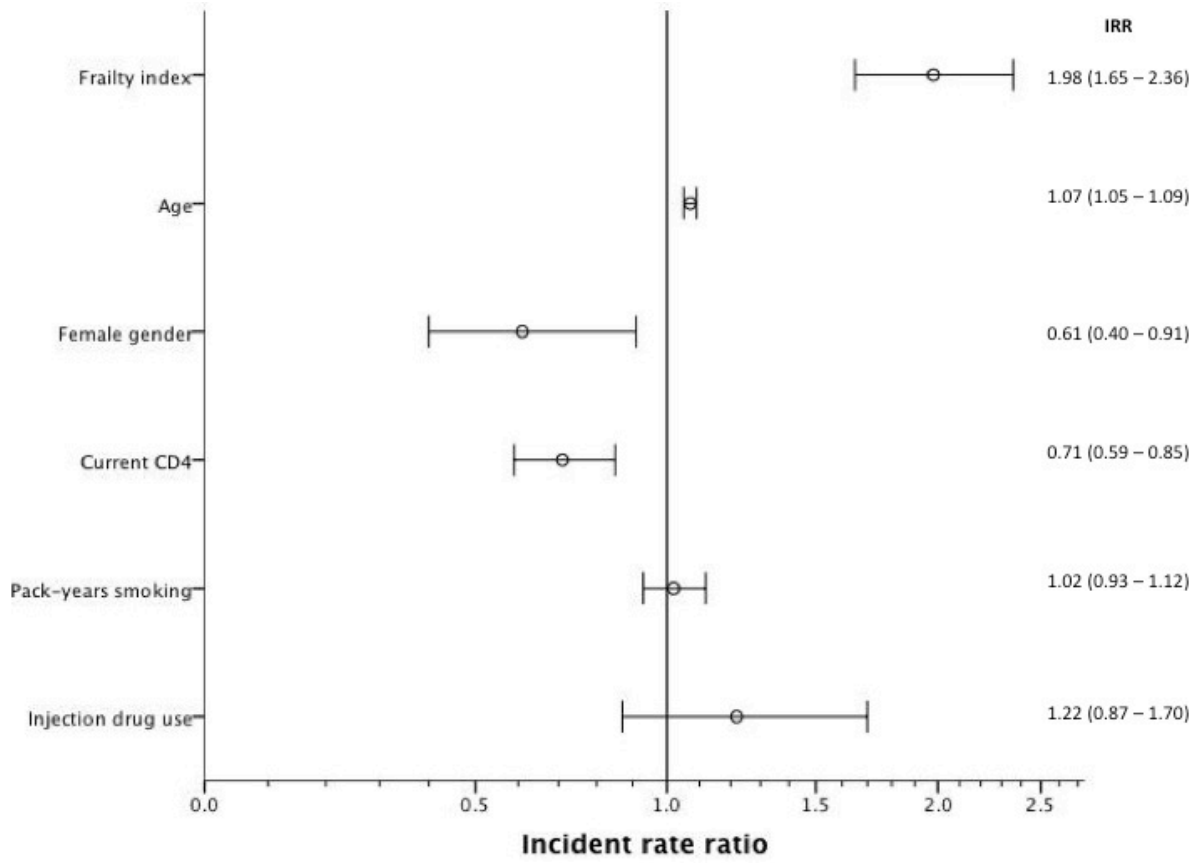

Figure 4. Predictors of incident multimorbidity in multivariate analysis. Points represent adjusted incident rate ratios, and whiskers 95% confidence intervals.

5. Ultrasound experience

Since September 2013 I've been working at the Infectious Diseases Unit of Reggio Emilia Hospital (ASMN-IRCCS), where sonography is widely used for both the evaluation of the critically ill patients and for diagnostic and interventional purposes.

Therefore I was trained in the use of clinical ultrasound, both diagnostic and interventional. Indeed, I learned abdominal ultrasound for the evaluation of liver, biliary system, pancreas, spleen, kidneys, bladder and bowel for both in and outpatients.

I have been performing contrast ultrasound for the evaluation of focal liver and splenic lesions by using a second generation contrast medium and a contrast harmonic imaging technology in selected patients.

Furthermore, I have been performing under sonograhic guidance biopsies (for diffuse and focal liver diseases, for lesions of the spleen and for lymphnodes), percutaneous ethanol injection and percutaneous thermal ablation with radiofrequency of hepatocellular carcinoma, abdominal abscesses drainage and central vein catheters positioning.

I have been performing bedside ultrasound for the evaluation of the critically ill patients (sepsis and septic shock), mainly for the evaluation of the hemodynamic state (by evaluating the lungs, the inferior cava vein and the heart).

As I had previous experience in clinical research in other fields, I applied my skills in investigating new insight in clinical ultrasound. Indeed, since I have been performing the aforementioned sonographic clinical activities, I participated to the publication of 2 papers about this topic.

a. "Evaluation with contrast ultrasound of the prevalence of splenic infarction in left-sided infective endocarditis".

We investigated the clinical significance of the contrast ultrasound of the spleen in 18 patients with definite left sided infective endocarditis.

AIM

The purpose was to prospectively evaluate the prevalence of the embolization of the spleen in patients with definite left- sided infective endocarditis (IE) using a contrast-enhanced ultrasound (CEUS).

Conclusions

In this study, CEUS of the spleen, a repeatable and low-cost imaging technique, easily allowed the bedside detection of asymptomatic and even tiny infarctions and showed a high rate of embolization in patients with definite left-sided IE. Therefore, in the setting of IE (possible or definite), CEUS of the spleen has the potential to better define or accelerate the diagnosis itself.

b. "Contrast-enhanced ultrasound (CEUS) appearance of hepatic myelolipoma".

Myelolipoma is a very rare benign hepatic tumor composed of fat and bone marrow components. It is more frequently located in the adrenal cortex. Indeed, myelolipoma of the liver is a very rare tumor; only a few cases are reported in the literature.
Myelolipoma of the liver was previously reported as hyperechoic lesions at sonography.

AIM

Describe the CEUS feature of hepatic myelolipoma

Conclusions

This is the first case reporting the CEUS features of hepatic myelolipoma in the literature. CEUS features, namely a homogeneous hyperenhancement in the arterial phase and a slight hyperenhancement in the portal venous phase, are typical of benign tumors. Focal nodular hyperplasia and high-flow hemangiomas may show this pattern of

enhancement. Therefore, the CEUS features of hepatic myelolipoma in this single case resulted attributable to a benign nodule and it should be considered in the differential diagnosis with other benign tumors. Only the biopsy of the tumor and the pathological evaluation allowed the diagnosis.

References

(1) Guaraldi G, Orlando G, Zona S, Menozzi M, Carli F, Garlassi E, et al. Premature age-related comorbidities among HIV-infected persons compared with the general population. Clin Infect Dis 2011 Dec;53(11):1120-1126.

(2) Schouten J, Wit FW, Kootstra N, van der Valk M, Geerlings SG, Prins M, et al. Cross-sectional comparison of the prevalence of age-associated comorbidities and their risk factors between HIV-infected and uninfected individuals: the AGEhIV Cohort Study. Clin Infect Dis 2014 Sep 2;pii: ciu701([Epub ahead of print]).

(3) Clegg A, Young J, Iliffe S, Olde Rikkert M, Rockwood K. Frailty in elderly people. Lancet 2013;381(9868):752-762.

(4) Rockwood K, Song X, Mitnitski A. Changes in relative fitness and frailty across the adult lifespan: evidence from the Canadian National Population Health Survey. CMAJ 2011 May 17;183(8):E487-94.

(5) Rockwood MR, MacDonald E, Sutton E, Rockwood K, Baron M, Canadian Scleroderma Research Group. Frailty index to measure health status in people with systemic sclerosis. J Rheumatol 2014;41(4):698-705.

(6) Fried L, Tangen C, Walston J, Newman A, Hirsch C, Gottdiener J, et al. Frailty in older adults: evidence for a phenotype. J Gerontol A Biol Sci Med Sci 2001;56(3):M146.

(7) Rockwood K, Mitnitski A. Frailty defined by deficit accumulation and geriatric medicine defined by frailty. Clin Geriatr Med 2011 Feb;27(1):17-26.

(8) Kulminski AM, Ukraintseva SV, Kulminskaya IV, Arbeev KG, Land K, Yashin AI. Cumulative deficits better characterize susceptibility to death in elderly people than phenotypic frailty: lessons from the Cardiovascular Health Study. J Am Geriatr Soc 2008 May;56(5):898-903.

(9) Theou O, Brothers TD, Mitnitski A, Rockwood K. Operationalization of frailty using eight scales and comparison of their ability to predict all-cause mortality. J Am Geriatr Soc 2013;61(9):1537-1551.

(10) Saum KU, Dieffenbach AK, Müller H, Holleczek B, Hauer K, Brenner H. Frailty prevalence and 10-year survival in community-dwelling older adults: results from the ESTHER cohort study. Eur J Epidemiol 2014;29(3):171-179.
(11) Mitnitski A, Song X, Skoog I, Broe GA, Cox JL, Grunfeld E, et al. Relative fitness and frailty of elderly men and women in developed countries and their relationship with mortality. J Am Geriatr Soc 2005 Dec;53(12):2184-2189.
(12) Streiner DL, Norman GR. Health measurement scales: a practical guide to their development and use. : Oxford University Press; 2008.
(13) Rockwood K, Rockwood MR, Mitnitski A. Physiological redundancy in older adults in relation to the change with age in the slope of a frailty index. J Am Geriatr Soc 2010;58(2):318-323.
(14) Womack JA, Goulet JL, Gibert C, Brandt CA, Skanderson M, Gulanski B, et al. Physiologic frailty and fragility fracture in HIV-infected male veterans. Clin Infect Dis 2013;56(10):1498-1504.
(15) Guaraldi G, Cossarizza A, Franceschi C, Roverato A, Vaccher E, Tambussi G, et al. Life expectancy in the immune recovery era: the evolving scenario of the HIV epidemic in northern Italy
. J Acquir Immune Defic Syndr 2014;65(2):175-181.
(16) Brothers TD, Rockwood K. Biologic ageing, frailty, and age-related disease in chronic HIV infection. Curr Opin HIV AIDS 2014;9(4):412-418.
(17) Erlandson KM, Schrack JA, Jankowski CM, Brown TT, Campbell TB. Functional impairment, disability, and frailty in adults aging with HIV-infection. Curr HIV/AIDS Rep 2014;11(3):279-290.
(18) Brothers TD, Kirkland S, Guaraldi G, Falutz J, Theou O, Johnston BL, et al. Frailty in people aging with human immunodeficiency virus (HIV) infection. J Infect Dis 2014;210(8):1170-1179.
(19) Desquilbet L, Margolick JB, Fried LP, Phair JP, Jamieson BD, Holloway M, et al. Relationship between a frailty-related phenotype and progressive deterioration of the immune system in HIV-infected men. J Acquir Immune Defic Syndr 2009 Mar 1;50(3):299-306.

(20) Althoff KN, Jacobson LP, Cranston RD, Detels R, Phair JP, Li X, et al. Age, comorbidities, and AIDS predict a frailty phenotype in men who have sex with men. J Gerontol A Biol Sci Med Sci 2014;69(2):189-198.
(21) Piggott DA, Muzaale AD, Mehta SH, Brown TT, Patel KV, Leng SX, et al. Frailty, HIV infection, and mortality in an aging cohort of injection drug users. PLOS One 2013;8(1):e54910.
(22) Ianas V, Berg E, Mohler MJ, Wendel C, Klotz SA. Antiretroviral therapy protects against frailty in HIV-1 infection. J Int Assoc Provid AIDS Care 2012;12(1):62-66.
(23) Pathai S, Gilbert C, Weiss HA, Cook C, Wood R, Bekker LG, et al. Frailty in HIV-infected adults in South Africa. J Acquir Immune Defic Syndr 2012;62(1):43-51.
(24) Terzian AS, Holman S, Nathwani N, Robison E, Weber K, Young M, et al. Factors associated with preclinical disability and frailty among HIV-infected and HIV-uninfected women in the era of cART. J Womens Health (Larchmt) 2009 /;18(12):1965-1974.
(25) Onen NF, Patel P, Baker J, Conley L, Brooks JT, Bush T, et al. Frailty and pre-frailty in a contemporary cohort of HIV-infected adults. J Frailty Aging 2014;3(3):158-165.
(26) Akgun KM, Tate JP, Crothers K, Crystal S, Leaf DA, Womack J, et al. An adapted frailty-related phenotype and the VACS Index as predictors of hospitalization and mortality in HIV-infected and uninfected individuals. J Acquir Immune Defic Syndr 2014 Sep 8;[Epub ahead of print].
(27) Marquine MJ, Umlauf A, Rooney AS, Fazeli PL, Gouaux BD, Paul Woods S, et al. The Veterans Aging Cohort Study index is associated with concurrent risk for neurocognitive impairment. J Acquir Immune Defic Syndr 2014;65(2):190-197.
(28) Fontana L, Kennedy BK, Longo VD, Seals D, Melov S. Medical research: treat ageing. Nature 2014;511(7510):405-407.
(29) Shannon CE. Von Neumann's contributions to automata theory. Bull Amer Math Soc 1958;64:123-129.

(30) Drubbel I, de Wit NJ, Bleijenberg N, Eijkemans RJC, Schuurmans MJ, Numans ME. Prediction of adverse health outcomes in older people using a frailty index based on routine primary care data. J Gerontol A Biol Sci Med Sci 2013;68(3):301-308.

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