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Learning and applying research skills in order to transfer them in different clinical settings: prospective cohort studies to build research capacity

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Table of context:

Summary (English)

The research work here presented was conducted with the aim of developing and transferring the skills and methodologies required to promote and enhance operational research capacity in differently resourced health care settings. The program has been built on three different intertwining components: first, theoretical learning of research methods, with focus on epidemiology and biostatistics; then the application of acquired skills to develop and conduct independent original research in collaboration with international research groups and finally, transferring the research skills either in coordinating and preparing a multicenter study in a resourced-limited setting or in analyzing, reviewing and editing works of other researchers.

The present thesis includes a summary of the competencies acquired at university of Padova and at the Harvard School of Public Health, reports the original research carried out by the candidate during the program and outlines the research activities in which the candidate was involved as coordinator and collaborator,
The three original research works were cohort studies (prospective or retrospective) on infectious disease epidemiology or progression (HIV and gastroenteritis).

The first and main original research work was a survival study realized on a multicenter cohort of HIV patients in a low-middle income country (Thailand) with an international research group. This prospective cohort's objective was to estimate survival at 5 years for patients with HIV treated with antiretroviral treatment (ART) and to identify the risk factors for mortality occurring immediately after starting ART (early mortality, defined as <6 months) and later during follow-up (long-term mortality, defined as >6 months). The rationale was that in resource limited settings, while early mortality is known to be high and caused by advanced disease status at the time of treatment initiation, much less is known on magnitude and predictors of long-term mortality in these settings. The candidate was involved in data collection, and was responsible for planning the study and its analysis, analyzing the data, interpreting the results and composing the corresponding paper and poster.

The second work was the analysis of a multicenter prospective cohort of HIV patients in Canada. The aim of the study was to identify which variables known at baseline could predict the occurrence of end stage liver disease in patients with HIV/HCV co-infection. The candidate planned the study and its analysis, analyzed the data, interpreted the results and along with co-authors, wrote the corresponding publications.
The third work was a retrospective cohort study, aimed to describe the epidemiology and management of a common infectious disease (acute gastroenteritis) at ambulatory facilities in Italy. The role of the candidate was to interpret and discuss results, as well as organize and collaboratively write the final paper.

Finally, during this program, the candidate was involved in skill transferring activities: specific aspects of research (for example literature review) for teachers and formal training on epidemiology for medical students in an university in resource limited settings, site investigator and coordinator of a multicenter study on HIV prevention in resources limited settings; collaborating in results interpretation and paper writing with units in a tertiary level hospital (Padova University).

In conclusion, in these years of doctoral training, the candidate acquired specific and technical competencies in research methodology, focusing on conducting and planning operational research in differently resourced settings in which medical care is provided. She further demonstrated how effectively her skills could be applied in various research environments, leading to the production of works either published, presented in international conferences or under review for publication. Finally, her skills were transferred to support the inception of new studies, leading to an enhanced research capacity in necessitous environments.

Riassunto (in italiano)

Apprendimento ed applicazione di metodologia della ricerca per trasferirla in diverse realtà cliniche: pianificazione, esecuzione ed analisi di studi osservazionali prospettici per costruire capacità di ricerca

Il lavoro di ricerca qui presentato è stato condotto con lo scopo d apprendere, applicare e trasferire abilità e metodologia necessarie per promuovere e potenziare la ricerca operativa in realtà sanitarie a diverso livello di risorse disponibili. Il programma è costituito dall'intreccio di tre diverse componenti : la prima è l'apprendimento teorico di nozioni di metodologia della ricerca, con particolare enfasi in epidemiologia e biostatistica; la seconda l'applicazione delle capacità acquisite nello sviluppare e condurre ricerca originale, collaborando con gruppi internazionali; la terza trasferire le capacità acquisite nel coordinare e predisporre uno studio multicentrico in un paese a risorse limitate, e nel supportare tramite analisi, revisione o edizione del lavoro di ricerca, altri ricercatori.

Questa tesi presenta un sommario di quanto appreso all'inizio del dottorato nell'Università di Padova e nella Harvard University (Boston, USA); riporta gli studi originali che sono stati fatti dal candidato durante il programma di studio, ed elenca le attività di ricerca nelle quali il candidato è stato coinvolto come coordinatore o collaboratore.
Gli studi principali, usati per consolidare particolari aspetti della metodologia, ritenuti uliti per esportare in ambienti diversi, sono stati studi di coorte (prospettici e retrospettivi), su epidemiologia o risposta alla terapia o progressione clinica delle malattie infettive (in particolare HIV e gastroenteriti).

Il lavoro di ricerca presentato per primo, ed il principale, è uno studio multicentrico di coorte, sulla sopravvivenza di pazienti con HIV, in un paese a basso-medio livello economico (la Tailandia) realizzato in collaborazione con un gruppo internazionale di ricerca. L'obiettivo d questa coorte prospettica ere quello di stimare la sopravvivenza a 5 anni di pazienti con HIV che iniziavano la terapia antiretrovirale e di identificare i fattori di rischio per mortalità dei primi 6 mesi (mortalità "precoce") e nei mesi successivi al 6° e fino a 5 anni (mortalità "a lungo termine"). Il razionale è che nei paesi in via di sviluppo, benché si sappia che la mortalità precoce è alta e dovuta ad uno stadio avanzato della malattia nel momento in cui si inizia la cura, sono meno note le dimensioni e rischi della mortalità a lungo termine, che avviene durante gli anni di trattamento. Il candidato è stato coinvolto in raccolta ei dati, e responsabile per pianificazione delle studio e dell' analisi, esecuzione dell'analisi, interpretazione dei risultati e scrittura dell'articolo e poster.

Il secondo lavoro è stato uno studio prospettico su una coorte multicentrica di pazienti con duplice infezione, da HIV e HCV, in Canada. Scopo dello studio per identificare le variabili conosciute

all’arruolamento nella coorte e che possono predire il verificarsi di malattia epatica terminale in pazienti coinfetti da HIV e HCV. In questo caso il candidato ha pianificato lo studio e l’analisi, condotto l’analisi, interpretato i risultati e scritto la pubblicazione corrispondente, assieme con i coautori.
Il terzo lavoro è stato uno studio osservazionale retrospettivo, per descrivere epidemiologia e gestione ambulatoriale della gastroenterite acuta nella popolazione pediatrica. Per questo lavoro il contributo del candidato è stato di interpretare e discutere i risultati ed organizzare, coordinare e scrivere l’articolo.

Infine, durante questo dottorato, il candidato è stato coinvolto in alcune attività di ricerca che hanno permesso di iniziare a trasferire le competenze apprese. Esempi sono un worskshop su come fare ricerca sistematica della letteratura esistente per gli insegnanti della facoltà di scienze mediche e corsi di epidemiologia per studenti della stessa, in un paese a baso reddito. Oppure aver iniziato e coordinato uno studio multicentrico sulla prevenzione di HIV nella stessa università; o aver collaborato a revisione di protocolli o interpretazione di risultati e scrittura di articoli con ricercatori dell’Università di Padova.

Concludendo, negli anni del programma di dottorato, il candidato ha acquisito competenze specifiche e tecniche in metodologia della ricerca, in particolare su come pianificare e condurre ricerca operativa in realtà atte a svolgere un servizio sanitario, a diversa disponibilità di risorse. Ha inoltre dimostrato come le sue capacità possano essere messe al servizio di diversi ambienti di ricerca , arrivando alla produzione di lavori pubblicati o in pubblicazione, e a presentazioni a conferenze internazionali. Non ultimo le sue capacità sono state utilizzate per promuovere una mentalità della ricerca ed iniziare nuovi studi, aumentando la capacità di conoscere e produrre ricerca in ambienti con queste necessità.

Introduction

Preface

Before becoming involved in this doctoral program, I completed my medical degree and residency in infectious diseases (ID) in Padova University, with main focuses on HIV (prevention, treatment and global patients care) and tropical medicine.

The work experience following the ID residency and preceding the doctorate brought me in a middle-income country, Thailand, in a consortium of international researchers working on HIV prevention studies and proving antiretroviral treatment.

The experience I acquired, both during my residency and later in Thailand, led me to realize how implementing research can lead to tangible improvements in medical services provided both in resource rich and limited settings. For example, at the pediatric clinic for children with HIV (Pediatric Department, Padova University), participating in a site for multicenter clinical trials on treatment and other aspects of pediatric HIV medicine (http://www.pentatrials.org/network.htm) allowed the patients to gain access to the latest treatment and prevention strategies, and sparked discussion on the quality of life of children with HIV and their families, leading to the assessment of novel approaches of psychological assistance.

Even more strikingly, in a low-middle income country like Thailand, research on the prevention of vertical HIV transmission, realized in public hospitals thorough the country, was able to improve the quality of care provided at the sites and at the same time provide the basis for national and international change in therapeutic guidelines for pediatric HIV (Lallemant et al. NEJM 2004).

Experience in these two realities led my interest closer to the research world, and strengthened my objective to become a health professional to implement and promote spontaneous research in settings dedicated to care. Research could be used both as an impetus to improve the quality of care provided and as a tool to obtain critical information and answer questions raised at the patient care level. The work presented here represents the results moved by these intentions, with the aim to contribute to realities with limited resources or to clinical settings with limited research capacity in order to enable effective clinical research works.

Introduction

The current doctoral program began with the aim of translating from the medical care mentality to the researcher's one. In order to do so, acquiring new skills was needed, as well as experience to apply them, in order to later conduct and promote independent research. The competencies were to enable the candidate in becoming responsible for clinical research in settings where there was little other expertise in the field. In particular, the interest was to acquire techniques that could have been applied not only in another tertiary care level setting (as Padova University Hospital), but also in more remote or less resourced settings. In order to do so, the program was carried out with different international collaborations, which allowed for experience with different realities of care.

Given this objective, the most appropriate technical knowledge and practical experience in methodology of clinical research to acquire were considered to be in operational research and observational studies. In order to eventually transfer capacity to health workers in rich or limited resources settings, theoretical knowledge would have to be consolidated through application in data analysis and operational research studies in various clinical realities.

This doctoral program thus followed a path through different university and clinical care settings, with the common aim to acquire, apply and transfer research skills applicable to answer research questions at the clinical level. Following the completion of the program, the candidate will be able to use the experience acquired to establish or strengthen research capacity in clinical settings with any level of resources.

Why clinical research?

While preclinical research is essential to develop mechanisms and for diagnosis and treatment of diseases, clinical research is necessary to confirm, apply and utilize what has been discovered. There are different types of clinical research, all within the intersection of medical practice and fundamental research. Clinical research, when applied in clinical sites, can further patient care in different ways. On one hand, it provides evidence for best practice, once the studies are concluded and validated. On the other hand, it improves quality of care during the collection of high quality data, while applying standard operating procedures (SOP) or when a study site is receiving specific training on one of the study's topics.

In resource-limited settings, operational research is a way to test and improve the health services in place. Currently, many international agencies are undertaking efforts to enhance and build capacity for operational research in such settings. One such example is the recent collaboration

between “International Union Against Tuberculosis and Lung diseases” and Médecins Sans Frontières (MSF) to implement and promote operational research (http://www.theunion.org).

Different research needs are specific to countries with limited resources. On one side is the need for research on specific diseases and conditions not common in other settings, such as neglected diseases. On the other is the important aspect of research in health systems, identified as at the driving force to improve the delivery of care, trying to maximize efficacy of the scarce resources available. Both are recognized as priorities by the WHO (http://apps.who.int/tdr/svc/research; http://www.who.int/rpc/publications/scaling_up_research/en/index.html).

In addition, research in developing countries can provide the contextual knowledge essential for research to have an impact on improvement of care settings, as it tells how the results of previous research can be applied to a specific population or country. While interventions can be proven effective in previous research, testing them in specific populations is essential, as is researching their usefulness, appropriateness and applicability given the health care resources, processes and structures in a given country (Bloom BR et al, in Disease Control Priorities in Developing Countries, ed World Bank, 2006). Therefore, thanks to operational research, discoveries of basic research can have their application supported by necessary evidence.

In many clinical settings, where health services are provided to in- or out-patients, operational research can bring additional value to the care provided, making use in prospective studies of the data collected during clinical follow-up. However, in settings where the main aim is patient care, time and capacity to conduct research are often lacking, with little research being done outside of clinical trial settings. This is especially true in resource-limited settings, where health workers are usually already overwhelmed by significant caseloads.

Acquiring competencies in research methodologies

In order to acquire specific technical skills in an environment suitable to learning research methods while exchanging with public health researchers with international experience, the candidate completed the degree of Master in Public Health (MPH, Harvard School of Public Health, Boston, MA, USA). The formal, one-year training is an intensive course on public health, comprised of theoretical lectures, seminars and group activities, with contents directly aligned with the overall objectives of the doctoral project.

The subjects studied included Biostatistics (methods of general biostatistics and logistic regression), Analysis with SAS, Methodology of Clinical Trials, Research in Developing Countries (ethical and operational issues), Epidemiology (general and advanced principles in epidemiology); Epidemiology of HIV (prevention and treatment studies), Society and Health, Ethics in Public Health, Management and Policy in Public Health, International Health and Environmental Health.

The rationale behind the biostatistics courses was to earn statistical skills useful to exchange with biostatisticians and to independently plan and conduct analysis in future studies (sample size calculation, choice of appropriate statistical tests, etc.). Epidemiology training focused on the interpretation of collected and published data (in particular on HIV medicine), in order to organize future studies and to support quality data collection at different levels of a health system, from rural health posts to tertiary centers. Further, the ability of critically reading existing literature is fundamental to conduct literature reviews and this research skill can be transferred to health care workers to improve medical care and use in their professional development.

Courses on research in resources-limited settings, international health and ethics in public health strengthened the candidate's awareness of the challenges of performing research in developing countries and provided meaningful exchange on strategies to overcome some of these barriers.

Acquiring proficiency in statistical analysis packaged such as Stata and SAS programs was useful to independently perform most of the analysis plans in subsequent research works. In addition, knowledge of the two most utilized statistical programs can be an asset to contribute to many research teams. Understanding the logic behind SAS and Stata allows future learning of other statistical programs in a more efficient way (such as R, a free online package utilizable in resource-limited settings).

Moreover, epidemiology and research methods were presented in different contexts, and with particular attention to technical and ethical issues of producing research in developing countries. For example, key observational and intervention studies done in developing countries were studied and critically appraised, in light of contextual methodological and ethical issues. Often, the realization of the studies, along with their impact at international and local levels was debated with the authors themselves.

Lastly, to follow the objective of integrating research in clinical settings, the above courses have been taken within the specific entity of Harvard's MPH program. Indeed, this program also stressed the important link between knowledge of quantitative methods and its impact on health systems functioning. The research methodology has therefore been learnt in the context of how results of medical studies could be utilized to inform not only practitioners, but also policy makers.

Observational cohort studies as a means for skills application

The second and central phase of the program was centered on applying the acquired competencies. This enabled the candidate's medical background to evolve progressively to that of

an independent researcher by employing research skills through collaboration with experienced researchers.

Observational cohort studies were chosen as the most useful to meet the objective of this doctoral project. Currently randomized clinical trials are considered worldwide the gold standard in clinical research to gather evidence on which new drugs utilization, prevention strategy or treatment guidelines can be designed. However the realization of clinical trials require substantial resources (financial, material and human). In addition, even where resources are available, clinical trials are not always a feasible choice. Prospective cohorts, even in realities where huge investments for clinical research are not available, can be a way to gather good quality data to be utilized for descriptive and analytical studies. In addition, when the data needed are impossible to be found in a unique cohort, through collaboration among different one and sharing of data, questions that could not be addressed in only one cohort can be studied. More and more international collaborations arise to share and utilize data collected in prospective cohort studies, both in developed and developing countries. Many examples are available in the field of HIV medicine, such as ATCC (in Europe and North America), IeDEA (Americas, Asia, Australia, Africa) or Treat-ASIA (Asia and South-east Asia) (http://www.art-cohort-collaboration.org; http://www.iedea-hiv.org/; http://www.amfar.org/treatasia), among many others.

Enhancing research through cohorts is an appealing option to implement in many of clinical settings, where data collected for clinical follow-up also becomes useful to perform research. Observational research lends itself well to settings with limited resources and where the only training and resources are the one for clinical care. Also, once an observational cohort has been established, it can be utilized to address different research questions. The data are valuable to reject hypothesis and/or to describe the epidemiology of a diseases and at the same time, provide and promote standards of care.

Additionally, the choice of practicing on observational cohorts studies allowed to gain operational experience, since these established observational cohorts allowed to learn how to reach high standards of data collection and analysis in differently resourced settings.
Conditions to perform observational studies are not always readily available in most clinical care settings, since appropriate data collection requires resources an expertise to enable a high level of quality of data extraction, collection and management and careful advanced planning to allow valid analysis. Steps needed to prepare the setting to prospective observational research include training of personnel involved in data collection, planning which type of data to collect, the type of form in which can be collected (software or hard copy) and involving the site investigators.

Significant experience was also gained in data analysis. Specific issues arise from cohort patients at the analysis step, such as missing data or different length of follow-up. Compared to clinical trials, this represents more a “realistic” situation, similar to what takes place in clinical care settings.

It was of considerable value to learn how to troubleshoot some of these analysis challenges, such as the technique for the imputation of missing baseline values used in the survival study.

Choice of collaborative research centers

The choice of different clinical settings was done in order to be able to reproduce spontaneous medical research in various realities in the future. These specific studies yielded key experiences on how data collection is to be organized in multicenter networks of hospitals or ambulatory centres with large numbers of patients in follow-up for chronic conditions or in primary care medicine.

The main study was conducted in collaboration with reputable international research team operating in Thailand (http://www.phpt.org). This is a unique situation where high quality research is being performed in a middle-low income setting – where research is more challenging than in Europe and North America, from limitations in infrastructure and man power. The team has realized clinical trials that are now milestones in the field of HIV vertical transmission, and therefore offers a milieu with high quality data and human expertise in the field.

The second study was performed in collaboration with the Canadian co-infection cohort (http://www.cocostudy.ca/co-infection_information.html). In this case it was an experience on a recently founded collaborations among public tertiary level hospitals in a resourced setting. Even still regarding HIV medicine, it gave the possibility to focus in different and more specific issues; as the long term consequences of co-infection with HCV. In addition, the population followed (sex, age, route of transmission, etc) and the treatment available (for HIV and especially for HCV) were different, and the types of data collected. This exposed the candidate to additional methodological issues and experience, as for example to deal with more sophisticated outcome as “end stage liver disease” diagnosis.

The third research component had the particularity of being conducted at primary care level, with a dynamic cohort of patients consulting primary pediatricians for a common disease (gastroenteritis) (www.pedianet.it). This was interesting to witness the possibilities and the challenges of conducting research using retrospective cohort data collected by primary care practitioners. One of the constraints of realizing cohorts for research is the ability of finding the balance with amount of data that is possible to collect in a primary care setting, without increasing too much the workload of physicians and nurses.

Experience gained through knowledge application in research

The application phase of the doctoral program allowed the candidate to earn substantial experience in various aspects of research.

In particular, the studies first exposed the candidate to the necessary use of different, context-specific methodologies. Examples of methods used for data analysis and results interpretation include techniques in survival analysis, multiple imputation of missing data, logistic regression and programming in statistical software as Stata and SAS. These were all direct applications of the knowledge learned in the biostatistics training, applied in order to realize actual analyses.

A second result of conducting this research work was a notable gain in experience and ability to critically discuss a study and analysis plan, through intimate involvement in planning the objectives and analysis of the different studies. These were valuable applications of epidemiology, progressing to applied levels beyond the training acquired in the MPH course.

Third, the candidate acquired experience in literature review. It is a fundamental research skill, which studies the current literature on the related topics. The candidate performed this before planning the analysis of the studies and during their realization. The candidate also earned by drafting a chapter on HIV in pediatric care and on various articles lead by other authors on HIV treatment. This experience is useful and versatile for future implementation of research projects.

Last is the noteworthy experience acquired in exchanging on research results with different teams and presenting them in international forums, which the candidate was able to do during co-investigators meetings or will be at the CROI conference (February-March 2011). Intimately linked to this is the additional benefit of a consolidating the candidate's level and knowledge of oral and written English, which grants access to platforms to share future results and ideas in the scientific international community.

Transferring research capacity

In the third and final stage of this doctoral project, the aim was to transfer the expertise acquired in order to enhance research capacity in settings with patients care, through collaborations with other professional in clinical or university settings. Such transfer can take many forms, from formal training, to activities oriented to mentoring others in the realization for specific research projects.

Research skills transfer was first experienced in a clinical setting unit through the collaborations in department of Pediatrics of the University of Padova. The candidate served as a scientific consultant for physicians needing to exchange ideas on research proposal, results interpretation

and paper writing. Needs raised by clinicians focused on methodology of study conduction and biostatistical support for analysis planning or execution or interpretation of results.

The candidate also transferred research expertise in a resource-limited setting, at Divine Word University (DWU), Papua New Guinea, in a series of collaborations. This took the form of her delivering two formal undergraduate training in epidemiology and research methods (of 45 hours each) in the Bachelor of Rural Health program. The candidate further developed the research capacity of the Faculty of Health Sciences at DWU by co-authoring a 4 days workshop on literature review methods for its lecturers and researchers, in collaboration with the Nossal Institute of Global Health, University of Melbourne. Last and most importantly, the candidate coordinated the opening of a research site at DWU for a multicenter survey on HIV prevention in Papua New Guinea, in collaboration with the School of Public Health, Tropical Medicine and Rehabilitation Sciences, James Cook University, Australia. This brought the candidate to coach the realization of the survey, deliver specific training to research workers and data collectors on HIV prevention, and to mentor site coordinators on many practical issues. Further, the candidate contributed to establishing the appropriate ethical considerations for the research, to the realization of data collection tools as well as to forming of the analysis plan, presentation of the study to stakeholders, and the logistics of its realization.

Research Activities I. Original research

In this section 3 original research works done by the candidate are presented. Each of them has resulted in a presentation to an international conference and / or a main article submitted to international journals and under peer revision, in which the candidate is the first author.

The main work has been the survival analysis for patients with HIV starting antiretroviral therapy, work presented in details in the following section.

Survival analysis of patients with HIV starting antiretroviral therapy: risk factors for early (0-6 months) and long-term (6 months to 5 years) mortality.

Preface:

This work has started during the second year of the program, and was carried out during the whole duration of it. Data were collected at a research site in Thailand, where the candidate had been working previously and periodically updated with the last data entered so that a longer follow-up time was available for the final work. Preliminary results of a first version of the analysis have been presented at: Symposium "Treating People with HIV: Research, Implementation, Public Health", on 24-26 November 2008, Chiang Mai, Thailand; partial results of the final analysis will be presented at the international Conference on Retroviral and Opportunistic Infections (CROI), in Boston, MA, USA (27 February- 3 March 2011).

The work has been submitted as original research paper to the Clinical Infectious Diseases journal and is under revision. The authors of the paper, as listed in the abstract accepted for the CROI and in the paper under revision, are:

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Introduction

In 2008, an estimated 4.7 million HIV-infected persons received antiretroviral treatment globally, of which approximately 85% were in resource-limited countries (1). The scale-up of access highly active antiretroviral therapy (HAART) is expected to continue , as only 42% of persons in clinical need of treatment are receiving it (1).

As national treatment programs mature, it is vital to evaluate their effectiveness and impact on long-term survival and retention. A number of studies have reported higher mortality during the first 6-months to one-year of therapy in resource-limited settings as compared to resource-rich countries (2-5). This has been attributed to late initiation of therapy at advanced disease stage and difficulties in diagnosis and management of co-infections (5). Much less is known about the longer term survival on HAART in resource-limited settings. Furthermore, it is unclear if patients initiating therapy at advanced disease stage remain at higher risk of mortality in the long-term, or if other factors dominate – such as early immune and virologic response to therapy. Such data are important in informing clinical practice and policy.

This study estimated the survival at up to 5-years of HAART of previously untreated adults receiving treatment in a network of public hospitals throughout Thailand as part of the national scale-up efforts (6), reaching 200,000 patients on treatment in 2008 (7). Participants were enrolled prospectively in an observational cohort. We assessed factors associated with Early mortality (deaths within 6-months of HAART initiation) as compared to Long-term mortality (deaths after 6-months).

A review of the literature of studies on survival for patients starting antiretroviral treatment in resources limited settings, and in Europe and North America gave the following results:

- Few studies conducted in resources limited settings present data with a long follow up (at 2 years or longer)
- Mortality is higher in resource limited settings as compared to rich settings

- Mortality is the highest in the early period after starting treatment.
- Low CD4, low total lymphocytes, anemia, low BMI are among the common factors associated with early mortality in resources limited settings.
- CD4 and viral load values updates after months from ART initiation (4 or 6) are associated with survival at long-term, both in resources limited and not-limited settings.

The following table summarizes the results of review of literature on risk factors for mortality in patients on ART in different settings.

Studies on survival of adults on HAART, with specified main outcome.
All articles refer to patients treated with ART as previously naive, if no otherwise specified (see comments).
Abbreviations: ART= antiretroviral treatment, FU= follow-up; LTFU = Lost to follow-up; Hb = hemoglobin.
Specific references are reported after the table.

STUDY	Patients and Follow-Up (FU)	Probability of survival/Mortality rate	PROGNOSTIC FACTORS	COMMENTS
De Beaudrop 2008, Senegal	404; FU: (median, IQR) 46 (32-57) months	Calculated rate: 6.2 per 100 PY, from All-cause deaths: 93 Follow-up: 17,980 person/months of observation.	HIV mortality: -whole follow up: baseline CD4, BMI, Hb. -Early (≤ 6 months) baseline BMI and TLC; - Late (> 6 months): Baseline CD4 and Hb; 6th months VL	
Bisson, 2008, Botswana	410 pts, FU 1 year (317 patient-years)	Lost to follow up: 68 (16.6%, 13.1–20.5) before tracking and 22 (5.4%, 3.4–8.0) after Dead: 29 (7.1%, 4.7–10.0) before and 69 (16.8%, 13.3–20.8) after Alive on HAART: 313 (76.3%, 71.2–80.3) before tracking and 319 (77.8%, 73.5–81.7) after	Higher adjusted Hazard ration for male sex and baseline CD4<50	88% naive Study on active tracking of a sample of patients classified as LTFU and seeing how survival and hazard is changing before and after the tracking. Conclusion: mortality is underestimated if not considering LTFU.
Bozzette 2008, US (Veterans hospital)	Pts: 41,213 Average of 4 years (168,213 person-years)	All cause mortality: - 20.9 deaths per 100 patient/years of observation in 1995 - 5.2 deaths per 100 patient/years of observation in 2003	Adjusted HR for death 0.18 (95% confidence interval: 0.15 to 0.23) at 72 months of exposure to HAART.	Mortality before and after starting HAART. Retrospective cohort (Jan 1993 to Dec 2003).
Brinkhof 2008, Pool of cohorts in resources limited settings (ART LIN);	5,941 pts; 6 months	Lost to follow up: ➢ 211 patients (3.8%) not seen after the ART initiation visit, ➢ 880 (16.0%) LTFU later on ➢ 141 (2.6%) died in the first 6 months	Recent calendar year, low or missing CD4 at baseline, older age and advanced clinical stage.	Outcome : loss to follow up and loss to follow-up and death in the first 6 months

STUDY	Patients and Follow-Up (FU)	Probability of survival/Mortality rate	PROGNOSTIC FACTORS	COMMENTS
Johannessen 2008; Tanzania	320 patients median 10.9 months (IQR 2.9–19.5)	Mortality: ➢ 19.2 at 3 months, ➢ 29.0 at12 months ➢ 40.7% at 36 months	-Severe anemia (hemoglobin <8 g/dL; adjusted hazard ratio [AHR] 9.20; 95% CI 2.05–41.3) -Moderate anemia (hemoglobin 8–9.9 g/dL; AHR 7.50; 95% CI 1.77–31.9), - Thrombocytopenia (platelet count <150 × 109/L; AHR 2.30; 95% CI 1.33–3.99) - Severe malnutrition (body mass index <16 kg/m2; AHR 2.12; 95% CI 1.06–4.24).	Reliable CD4 cell counts were not available
Marazzi 2008; Mozanbique, Tanzania, Malawi	3456 pts Results reported at 1 year.	260 deaths (97/1000 PY) Mortality peaking in the first 3 months	CD4<200; Hb, BMI	
Toure 2008; Ivory Coast	10,211; 7.7 months (IQR 2.6–15.5)	At 18 months: ➢ probability of death 0.15 ➢ probability of being LTFU 0.21	low CD4, low BMI, low hemoglobin, advanced clinical stage, old age and poor adherence, male sex, attending a recently opened clinic (vs experienced)	
Ferradini 2007; Cambodia	416; median 23.8 months	350 (84.1%) still on HAART, 53 (12.7%) died, 6 (1.4%) transferred, 7 (1.7%) lost to follow-up. Estimates of survival: 0.87 at 12 months [95% CI 0.83–0.90] 0.85 at 24 months (95% CI 0.81–0.88)		95.2% were ART naive. 35/53 deaths (66.0%) occurred within 6 months after HAART initiation (median time to death 3.6 months)

STUDY	Patients and Follow-Up (FU)	Probability of survival/Mortality rate	PROGNOSTIC FACTORS	COMMENTS
Moh 2007; Ivory Coast	792; median 8 months	Incidence of mortality/100 person-year per pre-ART CD4 cell count: ➢ <200, 5.0 [95% CI 2.6–8.7] ➢ at 200–350 : 1.7 (95% CI, 0.6–3.8) ➢ >350 cells/ml: 0.0 (95% CI, 0.0–3.4]	- At baseline: severe morbidity, high viral load, advanced clinical stage, past history of tuberculosis, low BMI, low haemoglobin, low CD4 cell count; - During follow-up: low CD4 cell count , persistently detectable viral load.	
Etard 2006, Senegal	404 pts, median 46 months (17,980 persons-months observation)	Overall rate of death: ➢ 6.3/100 person-years [95% CI 5.2–7.7] In the first year: ➢ 11.7% (95% CI, 8.9–15.3%). At 2 years (cumulative probability): ➢ 17.4% (95% CI, 13.9–21.5%) At 5 years: ➢ 24.6% (95% CI, 20.4–29.4%)	Independent predictors in multivariate COX models: CD4 <200, Hb<10 and BMI <19	Very long (7 years) FU; Mortality trend changes with time.
Ferradini 2006, Malawi	1,308; Median 8.3 months (IQR 5.5-13.1)	At 12 months, probability to be still in care: 0.76 (95% CI 0.73-0.78)	Determinants of death in the first 6 months : -Low body-mass index, WHO stage IV, male sex, baseline CD4 count lower than 50 cells/mm3	97% HAART-naive,
Lawn 2006, South Africa	1235 patients screened of whom 927 pts started ART (1 year) 170 PY pre-ART and 808 PY during treatment	Deaths before starting ART: ➢ 33.3 deaths/100 person-years (95% CI, 25.5–43.0) In the first 4 months of ART (Early): ➢ 19.1 deaths/100 person-years; (95% CI, 14.4– 25.2) 4 months to 3 years of ART (Late): ➢ 2.9 deaths/100 person-years; (95% CI, 1.8–4.8) (After 1 year of ART: 1.3 deaths/100 person-years (95% CI 0.4–3.9)	- All FU deaths: baseline CD4 cell count and WHO clinical stage - Early (0- months): WHO stage, CD4 and male sex - Late: CD4 and VL at 4th month.	Risk of death decreasing with time, risk of LTFU constant. Risk factors of early and late deaths are different.

STUDY	Patients and Follow-Up (FU)	Probability of survival/Mortality rate	PROGNOSTIC FACTORS	COMMENTS
May 2006; Europe and North America	22,217	Compared with 1998, adjusted hazard ratios for death: ➢ 0.87 (0.56-1.36) in 1995-96 ➢ 0.96 (0.61-1.51).in 2002-03.		*"Virological response after starting HAART improved over calendar years, but such improvement has not translated into a decrease in mortality*." Review of 12 cohort studies
Palella, 2006, US (10 cities)	6,945; median 39.2 months.	702 deaths. Rates: from 7.0 deaths/100 person-years of observation in 1996 to 1.3 deaths/100 person-years in 2004 (P = 0.008 for trend)		HAART use: 43% in 1996 to 82% in 2004
Braitstein (ART-LINC) 2006; high and low income countries (comparison);	4,810 (with a baseline CD4),; 1 year FU.	Low income**:**124 deaths during 2236 person-years of follow-up). Mortality rates per 1000 person-years: 147 (95% CI 105–207) at 1 month, 106 (71–160) at month 2, 51 (33–77) at months 3–4, 51 (33–79) at months 5–6 27 (19–40) at months 7–12. High income settings: 414 deaths during 20 532 person-years. Early: 24 (21–27) the first 6 months Late: 16 (14–19) during months 7–12.	Free access to ART protective factors	1st outcome: mortality from all causes 2nd : Changes in CD4 cell counts, proportion of patients with viral load less than 500 copies/mL (6 months)
Laurent 2005; Senegal	176; 30 months IQR 21-36	Survival probability: ➢ 0.84 (CI 0.77–0.88) at 2 years ➢ 0.81 at 3 years; 95% CI, 0.74–0.86	Baseline hemoglobin level and Karnofsky score	92% of patients were antiretroviral naive

STUDY	Patients and Follow-Up (FU)	Probability of survival/Mortality rate	PROGNOSTIC FACTORS	COMMENTS
Coetzee 2004, South Africa;	287 pts; median 13.9 months.	Survival at 24 months: ➢ 86.3% (95% CI, 81.7–89.8%) at 24 months for all patients, ➢ 91.4% (95% CI, 84.9–95.1%) for those with a baseline CD4 lymphocyte count ≥ 50 cells/mm3, ➢ 81.8% (95% CI, 74.7–87.0%) for those with a baseline CD4 lymphocyte count <, 50 cells/mm3.	- Low CD4 lymphocyte count (AJR of death or loss to follow-up 2.41 (95% CI, 1.20–4.87) - previous diagnosis of Kaposi's Sarcoma (AJR of death or loss to follow-up 4.82 (95% CI, 2.12–10.97)	Limits: short duration of follow-up (number at risk is 34 at 24 months, and is less than 170 after the 1st year)
Seyler 2003; Ivory Coast	101; 17 months	Incidence of death ➢ 3.0/100 PY (1.1-8.0) in patients with baseline CD4 > or = 50/mm3 ➢ 16.1/100 PY (7.2-35.9) in patients with CD4 < 50/mm3.	Low CD4	
Egger 2002. Europe and North America (13 cohorts)	12 ,574; 24, 310 person-years	344 patients died; compared with patients starting with less than 50 CD4, adjusted hazard ratios : ➢ 0·74 (95% CI 0·62–0·89) for 50–99 cells/mm^3 ➢ 0·52 (0·44–0·63) for 100–199 cells/mm^3 ➢ 0·24 (0·20–0·30) for 200–349 cells/mm^3 ➢ 0·18 (0·14–0·22) for ≥350 CD4 cells/mm^3	Baseline CD4 cell count, VL, age, infection through IDU, previous diagnosis of AIDS	
Boulle 2010; South Africa	7323; FU up to 5 years (26% at 2 years; 2% reaching 5 years)	Corrected mortality (after matching LTFU with death registry): 9.9% at 1 year (95%CI 8.9 – 10.9) 12.6 % at 2 years (11.5 – 13.8) 20.9% at 5 years (17.9 – 24.3)	Low baseline CD4, AIDS diagnosis, low body weight: associated with first 3 months and beyond 3 months mortality; older age (>50 ys) associated with mortality after 3 months.	32.8% of patients LTFU were confirmed dead after matching with death registries.
Chene 2003; Europe and North America	9323; 13408 PY	152 deaths (1.1/100PY mortality rate)	Low CD4 and high viral load at 6 months. (Baseline CD4 and VL had no association with later mortality once corrected for 6 months values)	Analysis only on patients still on F at 6 months

STUDY	Patients and Follow-Up (FU)	Probability of survival/Mortality rate	PROGNOSTIC FACTORS	COMMENTS
Mugyenyi 2010; Uganda, Zimbabwe	3321, 4.9 years IQR 4.4 – 5.3) median FU	Survival at 5 years : 87%(95%CI 85-88) in clinical monitoring arm 90% (88 - 91) in laboratory and clinical arm		Clinical Trial (DART) comparing clinical *vs* clinical + laboratory monitoring
ATCC 2007, Europe and North America	20379, 61978 PY, median FU 3 (IQR1.5 – 4.5) years	1005 deaths, Mortality rates (95%CI): at 1 year 2/100PY (1.9 – 2.3) at 2 years: 1.5/100PY (1.3 – 1.7) at 4-6 years:1.5/100PY (1.2 – 1.8)	Baseline CD4 <25 as compared o >350 cells/mm^3 IDU as possible transmission route.	
Chasombat 2009, Thailand	58008 FU median 1.6ys (IQR 0.8 – 2.4)	Survival at 1 year : 0.89 (95%CI 0.88 – 0.89)	Male sex, age > 40ys, CD4 baseline <100 cells/mm3, AIDS, district/community hospital, year of enrollment < 2005.	
Tuboi 2009, Latin America	5152	1 year mortality rates: 8.3 (7.6 – 9.1)	Low CD4 baseline (<50 versus >200); AIDS	Data from a collaboration of 7 countries
Grabar 2005, France	2236, median FU 58 months, 8882 PY	161 deaths Rate of AIDS OR death: 3.37/100PY	CD4 and viral load at 6 months predictive of mortality/AIDS at 5 years	Analysis restricted to patients on FU at 6 months

References for above reported table of studies.

(2007). "Importance of baseline prognostic factors with increasing time since initiation of highly active antiretroviral therapy: collaborative analysis of cohorts of HIV-1-infected patients." J Acquir Immune Defic Syndr **46**(5): 607-15.

Bisson, G. P., T. Gaolathe, et al. (2008). "Overestimates of survival after HAART: implications for global scale-up efforts." PLoS ONE **3**(3): e1725.

Boulle, A., G. Van Cutsem, et al. "Seven-year experience of a primary care antiretroviral treatment programme in Khayelitsha, South Africa." AIDS **24**(4): 563-72.

Bozzette, S. A., C. F. Ake, et al. (2008). "Long-term survival and serious cardiovascular events in HIV-infected patients treated with highly active antiretroviral therapy." J Acquir Immune Defic Syndr **47**(3): 338-41.

Braitstein, P., M. W. Brinkhof, et al. (2006). "Mortality of HIV-1-infected patients in the first year of antiretroviral therapy: comparison between low-income and high-income countries." Lancet **367**(9513): 817-24.

Brinkhof, M. W., F. Dabis, et al. (2008). "Early loss of HIV-infected patients on potent antiretroviral therapy programmes in lower-income countries." Bull World Health Organ **86**(7): 559-67.

Chasombat, S., M. S. McConnell, et al. (2009). "National expansion of antiretroviral treatment in Thailand, 2000-2007: program scale-up and patient outcomes." J Acquir Immune Defic Syndr **50**(5): 506-12.

Chene, G., J. A. Sterne, et al. (2003). "Prognostic importance of initial response in HIV-1 infected patients starting potent antiretroviral therapy: analysis of prospective studies." Lancet **362**(9385): 679-86.

Coetzee, D., K. Hildebrand, et al. (2004). "Outcomes after two years of providing antiretroviral treatment in Khayelitsha, South Africa." AIDS **18**(6): 887-95.

De Beaudrap, P., J. F. Etard, et al. (2008). "Change over time of mortality predictors after HAART initiation in a Senegalese cohort." Eur J Epidemiol **23**(3): 227-34.

Egger, M., M. May, et al. (2002). "Prognosis of HIV-1-infected patients starting highly active antiretroviral therapy: a collaborative analysis of prospective studies." Lancet **360**(9327): 119-29.

Etard, J. F., I. Ndiaye, et al. (2006). "Mortality and causes of death in adults receiving highly active antiretroviral therapy in Senegal: a 7-year cohort study." AIDS **20**(8): 1181-9.

Ferradini, L., A. Jeannin, et al. (2006). "Scaling up of highly active antiretroviral therapy in a rural district of Malawi: an effectiveness assessment." Lancet **367**(9519): 1335-42.

Ferradini, L., D. Laureillard, et al. (2007). "Positive outcomes of HAART at 24 months in HIV-infected patients in Cambodia." AIDS **21**(17): 2293-301.

Grabar, S., V. Le Moing, et al. (2005). "Response to highly active antiretroviral therapy at 6 months and long-term disease progression in HIV-1 infection." J Acquir Immune Defic Syndr **39**(3): 284-92.

Johannessen, A., E. Naman, et al. (2008). "Predictors of mortality in HIV-infected patients starting antiretroviral therapy in a rural hospital in Tanzania." BMC Infect Dis **8**: 52.

Laurent, C., N. F. Ngom Gueye, et al. (2005). "Long-term benefits of highly active antiretroviral therapy in Senegalese HIV-1-infected adults." J Acquir Immune Defic Syndr **38**(1): 14-7.

Lawn, S. D., L. Myer, et al. (2006). "Determinants of mortality and nondeath losses from an antiretroviral treatment service in South Africa: implications for program evaluation." Clin Infect Dis **43**(6): 770-6.

Marazzi, M. C., G. Liotta, et al. (2008). "Excessive early mortality in the first year of treatment in HIV type 1-infected patients initiating antiretroviral therapy in resource-limited settings." AIDS Res Hum Retroviruses **24**(4): 555-60.

May, M. T., J. A. Sterne, et al. (2006). "HIV treatment response and prognosis in Europe and North America in the first decade of highly active antiretroviral therapy: a collaborative analysis." Lancet **368**(9534): 451-8.

Moh, R., C. Danel, et al. (2007). "Incidence and determinants of mortality and morbidity following early antiretroviral therapy initiation in HIV-infected adults in West Africa." AIDS **21**(18): 2483-91.

Mugyenyi, P., A. S. Walker, et al. "Routine versus clinically driven laboratory monitoring of HIV antiretroviral therapy in Africa (DART): a randomised non-inferiority trial." Lancet **375**(9709): 123-31.

Palella, F. J., Jr., R. K. Baker, et al. (2006). "Mortality in the highly active antiretroviral therapy era: changing causes of death and disease in the HIV outpatient study." J Acquir Immune Defic Syndr **43**(1): 27-34.

Seyler, C., X. Anglaret, et al. (2003). "Medium-term survival, morbidity and immunovirological evolution in HIV-infected adults receiving antiretroviral therapy, Abidjan, Cote d'Ivoire." Antivir Ther **8**(5): 385-93.

Toure, S., B. Kouadio, et al. (2008). "Rapid scaling-up of antiretroviral therapy in 10,000 adults in Cote d'Ivoire: 2-year outcomes and determinants." AIDS **22**(7): 873-82.

Tuboi, S. H., M. Schechter, et al. (2009). "Mortality during the first year of potent antiretroviral therapy in HIV-1-infected patients in 7 sites throughout Latin America and the Caribbean." J Acquir Immune Defic Syndr **51**(5): 615-23.

Methods

Study design and population: HIV-infected adults received antiretroviral therapy and were followed in a prospective observational cohort in public hospitals throughout Thailand, conducted by 'Programmes for HIV Prevention and Treatment (PHPT)' research group (NCT00433030 www.clinicaltrials.gov). The cohort began in 1999, recruiting women who participated in trials on prevention of mother-to-child transmission of HIV (8, 9); from 2003 enrollment was extended to partners of these women and any HIV-infected adult presenting at participating sites. From May 2005, a clinical trial comparing HAART monitoring strategies (PHPT-3 NCT00162682 www.clinicaltrials.gov) was nested in the cohort. Participants provided written informed consent at entry and the study was approved by the Thai Ministry of Public Health and local Ethic Committees. The criteria for entry to the cohort were confirmed HIV diagnosis, willingness to initiate therapy and to be followed-up in the program. The criteria for initiation of therapy were CDC stage C or CD4< 250 cells/mL (10). The inclusion criteria for this analysis were: antiretroviral naïve (except for PMTCT prophylaxis) and initiated with HAART (as opposed to dual therapy), defined as three or more drugs including at least two drug classes.

Antiretroviral treatment: Initial HAART regimens changed over time with improved availability of drugs: before 2003 unboosted Protease Inhibitor (PI)-based regimens were used, after 2003 most patients started a nevirapine based fixed-dosed combinations (GPOvir-Z© and GPOvir-S©) manufactured in Thailand, and after 2005 there was increased use of tenofovir, emtricitabine and efavirenz. Alternative regimens were provided as needed for toxicities or treatment failure. Patients with CD4 <200 cells/mm^3 received cotrimoxazole prophylaxis.

Follow-up: Patients attended the clinic monthly and had a basic physical exam, drug refills and adherence counseling conducted by a nurse. A physician saw them monthly in the first 3 months of treatment and 3-monthly thereafter and when referred by the nurse. CD4 and virology testing was done at start of HAART and every 3 or 6 months thereafter.

Loss-to-follow-up (LTFU) was defined as missed scheduled visit and no contact for over six months. Hospital staff actively traced patients with missed visits through telephone calls and home visits. Patients who informed the staff of leaving the cohort, often due to relocation, were considered as voluntarily withdrawn.

Data were collected prospectively at the hospital site by nurses or physicians on specifically designed Case Report Forms and sent to PHPT for data entry and management.

Outcomes and risk factors: The outcome of interest was all-cause mortality. Deaths were defined as "Early" if occurred ≤6-months after start of HAART or "Long-term" if occurred >6-months.

Events leading to death, and where available, the immediate cause and underlying conditions were reported by site physicians. All deaths were reviewed and causes of death were classified by two independent physicians based on the ICD-10 classification (http://www.who.int/classifications/icd/en/index.html). and on the CoDe coding (www.cphiv.dk/CoDe).

Factors considered for potential association with survival were sex and the following baseline characteristics at start of HAART: age, CD4 cell count, viral load, CDC stage, body mass index (BMI), hemoglobin level, calendar year, initial regimen as well as hospital size (number of patients in the study) and type of follow up (PHPT-3 trial or observational cohort). Time updated factors were: CD4, BMI, hemoglobin, viral load and treatment at 6-months after HAART initiation.

Statistical analysis: Mortality rates were calculated using Poisson regression, with follow-up time from the start of HAART until date of death or last visit. Kaplan-Meier probability of survival was estimated up to 5-years after start of therapy, differences in survival by risk factors were tested using the log rank test. Analyses were based on intent-to-continue treatment, ignoring treatment changes, interruptions or terminations.

Age, CD4 and viral load at baseline were used as continuous variables after testing for non-linearity using cubic spline method (11). Anemia, BMI and time updated CD4 and viral load at 6-

months after start of HAART were categorized guided by commonly used cut-offs in the literature (12-14). Calendar year of HAART initiation and hospital size were categorized guided by percentiles distribution in 3 categories (2002-3, 2004-5 and 2006-8), and 2 (small hospital <35 patients enrolled in the study) or large (≥35 patients) respectively. Note for calendar year: in some of the further analysis the first 2 categories (2002-3 and 2004-5) were lumped together as both had HR>1 (2006-8 was the referral category).

Cox proportional hazard models were first used to assess factors associated with overall mortality. The proportional hazard assumption was assessed using a formal test for interaction with follow-up time divided at before and after 6-months, based on the median time to death. Due to evidence of interaction with time ($p < 0.1$) for a number of covariates (data not reported), separate analyses were conducted for factors associated with Early and Late mortality. Baseline risk factors were assessed for association with Early mortality. Among patients alive and on follow-up at 6-months after start of HAART, baseline and time updated covariates were assessed for association with Long-term mortality. No major violation of proportionality assumption after the first 6 months were detected when tested graphically.

Covariates with associated p-value of ≤0.2 in univariate analysis were considered in multivariate analysis, added to the final model and kept, using forward selection, if associated p-value was <0.2. Age and sex were considered *a priori* as confounders and included in the final models. Interactions between gender and key risk factors were examined and $p<0.05$ was considered as evidence of interaction.

All variables had ≤7% of missing values. Nonetheless, to avoid loss of information and potential biased estimates due to missing data we used Multivariate Imputation by Chained Equations (MICE) method to impute all missing values for baseline and 6^{th} month covariates based on 10 cycles (15, 16), for the Cox univariate and multivariate analyses. Variables that have been used for the imputation of missing baseline values are: gender, age, CDC stage, baseline CD4 and viral load, hemoglobin, BMI. All these and 6^{th} month values of CD4, viral load, BMI and hemoglobin

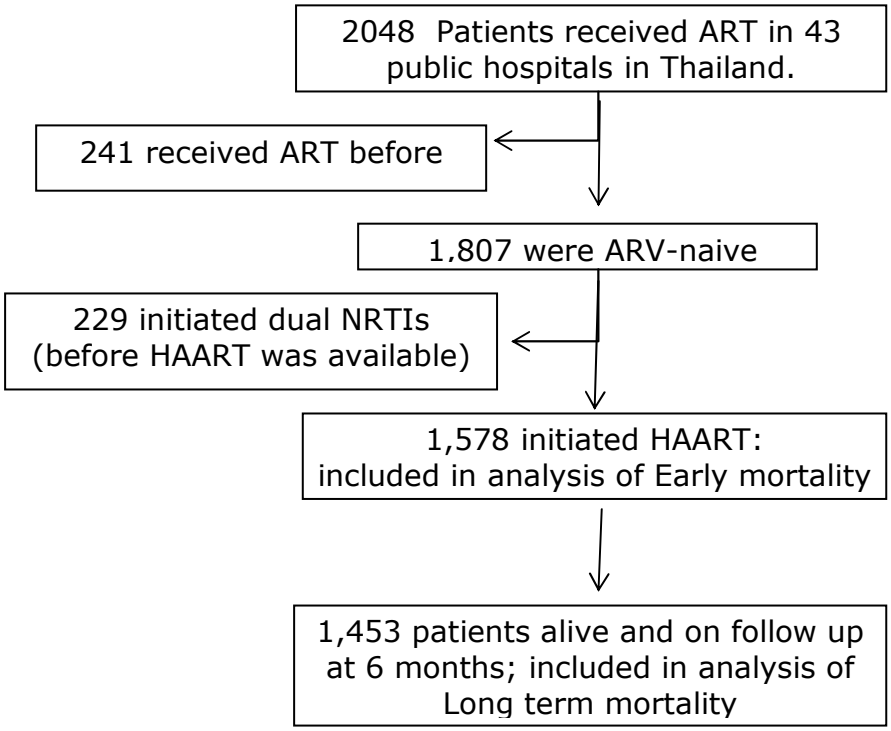
have been used for imputation of missing values at 6 months. Analysis gave similar results when using raw data instead of MICE.

Data were analyzed with STATA version 11 (Stata Corp., College Station, TX, USA).

Analysis and Results

A total of 2048 adults received antiretroviral therapy in the cohort, 241 were treatment experienced at entry and 229 started on a non-HAART regimen and therefore were excluded while 1,578 met the criteria for this analysis [Fig 1].

Figure 1: Flowchart of patients included in the analysis.



Legend: Early mortality: occurring between 0-6 months, Long-term mortality: between 6-60 months.

Patients started HAART between 2002 and 2008, 74% were women, the median (IQR) age at start of HAART was 33 years (28 to 38); median CD4 was 124 (57 to 196) cells/ml; 42% were in CDC stage B or C, 17% had anemia (hemoglobin <10g/dL) and 92% initiated with a NNRTI-based regimen (Table 1).

Table 1: Patient characteristics for entire cohort at HAART initiation and after 6 months of HAART – patients still on follow-up. Number (%), unless specified

At HAART initiation (n=1578)		At 6 months (n=1453)	
Sex			
Women	1170 (74)		
Men	408 (26)		
Age (years)	*n=1576 (99)*		
Median (IQR)	33 (28 – 38)		
BMI	*n=1558 (99)*	**BMI**	*n=1441 (99)*
≤18.5	369 (24)	≤18.5	235 (16)
>18.5	1189 (76)	>18.5	1206 (84)
CDC	*n=1504 (95)*		
A	876 (58)		
B	275 (18)		
C	357 (24)		
CD4 (cell/mm^3)	*n=1552 (98)*	**CD4** (cell/mm^3)	*n=1410 (97)*
Median (IQR)	124 (57-196)	Median (IQR)	245 (168 – 334)
>200	351 (23)	>200	933 (66)
>100 ≤200	545 (35)	100-200	358 (25)
>50 ≤100	306 (20)	50-100	77 (5)
≤ 50	350 (23)	≤ 50	42 (3)
Viral load (log_{10} copies/mL)	*n=1472 (93)*	**Viral load** (log_{10} copies/mL)	*n=1427 (98)*
Median (IQR)	4.9 (4.3 – 5.3)	≤ 1 log	1316 (92)
		> 1 log	111 (8)
Hemoglobin (g/dL)	*n=1510 (96)*	**Hemoglobin** (g/dL)	*n=1420 (98)*
> 10 (No anemia)	1247 (83)	> 10	1328 (94)
< 10 (Anemia)	263 (17)	≤ 10	92 (6)
Year enrollment			
2006-8	607 (38)		
2004-5	430 (27)		
2002-3	541 (34)	**Treatment at 6 months**	
Initial treatment		NVP based	676 (47)
NVP based	847 (54)	EFV based	608 (42)
EFV based	597 (38)	PI based	153 (10)
PI based	134 (8)	Temporary interruption	16 (1)
		CD4 change (cells/mm^3)	*n=1391 (96)*
Type of follow-up		Median (IQR)	114 (62 - 178)
Monitoring clinical trial	715 (45)	>200	261 (19)
Observational study	863 (55)	100 to < 200	523 (38)
Hospital (# patients within cohort**)**		50 to < 100	322 (23)
<35	342 (22)	≤50	285 (20)
≥ 35	1236 (78)	**Anemia at 6 months**	*n= 1381 (95)*
Mode of Entry		Never had	1121 (81)
Previous PMTCT trials	575 (36)	Recovered	171 (12)
Treatment program	418 (27)	New cases	35 (3)
Monitoring trial	585 (37)	Persistent	54 (4)

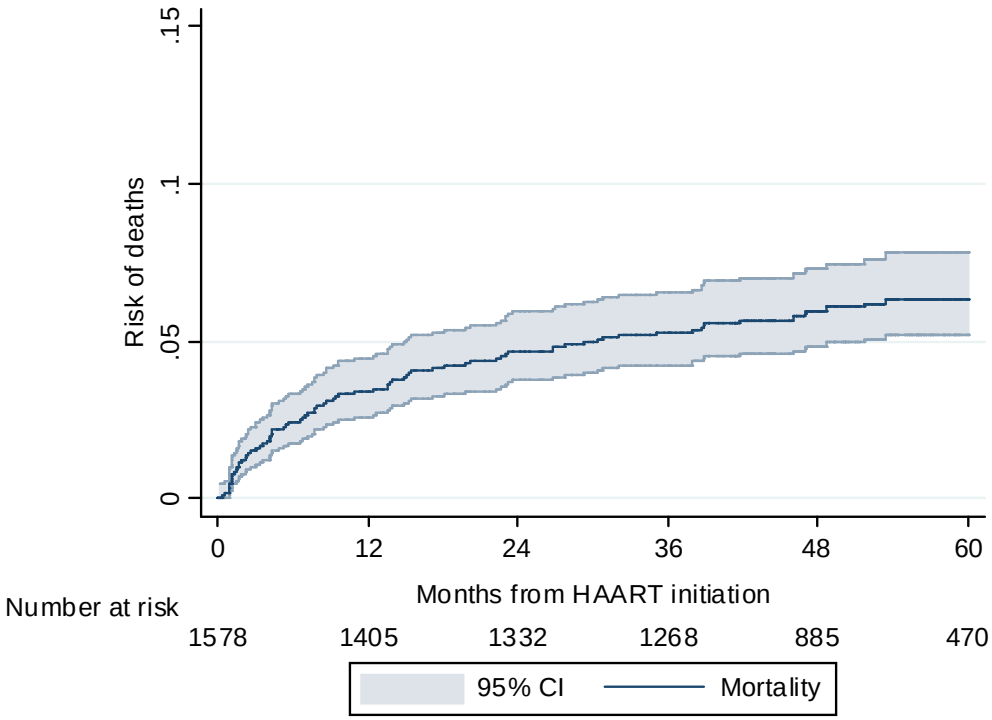
The baseline characteristics changed over time: patients enrolled from 2006 onwards were more likely to be men (37% vs 19%), less immunocompromised (8% vs 32% with CD4 <50 cells/mL) and less likely to be anemic (13% vs 20%) as compared to patients enrolled before then (all $p<0.05$). From 2006, first line treatment were more likely to be efavirenz-based regimens with tenofovir as compared to NVP or PI-based regimens before this date ($p<0.05$).

Follow-up and overall survival

There were 5,935 person-years of observation; the median duration of follow-up was 50 months (IQR 41 to 60) and 473 (30%) patients reached 5 years of follow-up after HAART initiation. There were a total of 89 (6%) deaths, 183 (12%) lost-to-follow-up and 142 (9%) voluntarily withdrew.

The Kaplan-Meier survival estimates (95%CI) were 97.6% (96.7-98.2) at 6-months; 96.6% (95.6-97.4) at 1-year and 93.7% (92.3-94.8%) at 5-years of HAART. Mortality was highest during the first 6-months of therapy, with 4.9 deaths (95% CI, 3.6-6.8) per 100 person years (PY). These early deaths accounted for 42% of the total deaths observed, with a median time to event of 1.9 months (1.1 – 3.9) after HAART initiation. After 6 months of HAART, 52 patients died (3.6%) and the mortality rate declined to 1.0/100 PY (95%CI, 0.8- 1.3) with a median (IQR) time to death of 19.0 months (10.4 to 31.4) (Figure 2a).

Fig2a. Kaplan Meier estimates of Mortality



Of the 89 deaths, 36% occurred in the hospital where patients were treated, 48% occurred at home and 16% in other hospitals.

The most common causes were AIDS defining events (26/77) and infections likely or possibly related to immunodeficiency (16/77). These two causes accounted for 54% (20/37) of early deaths and 42% (22/52) of long-term deaths.

Other causes were: 12 cardiovascular disease (2 myocardial infarction, 3 stroke and 7 others); 6 digestive system disease (3 liver failure, 1 gastrointestinal hemorrhage and 2 others); 4 suicide; 3 infections; 2 malignancy, 3 accidents (1 road, 1 in the hospital, 1 alcohol intoxication) and 1 of each for: severe anemia, central nervous system disease, respiratory disease, renal failure, lactic acidosis. Among the twelve unknown causes of death, ten occurred at home, and the latest CD4 was <200 cells/mm^3 in nine cases and within one year from starting treatment for six.

AIDS events, immunodeficiency-related infections and cardiovascular diseases were the most common causes for both early and late deaths.

Causes of deaths:

Diagnosis, n (%)	**Early (0-6 months)**	**Long-term (6-60 months)**	**Total**
AIDS related events*	20 (54)	22 (42)	42 (47)
Infections not AIDS-related	0 (0)	3 (6)	3(3)
Cardiovascular diseases	5 (14)	7 (13)	12 (13)
Gastrointestinal Diseases	3 (8)	3(8)	6 (7)
Malignancy	0(0)	2(4)	2 (2)
Accidents/suicide	2(5)	5 (10)	7 (8)
Others*	3 (8)	2(4)	5 (6)
Unknown	4(11)	8(15)	12 (13)
Total	37 (100)	52 (100)	89 (100)

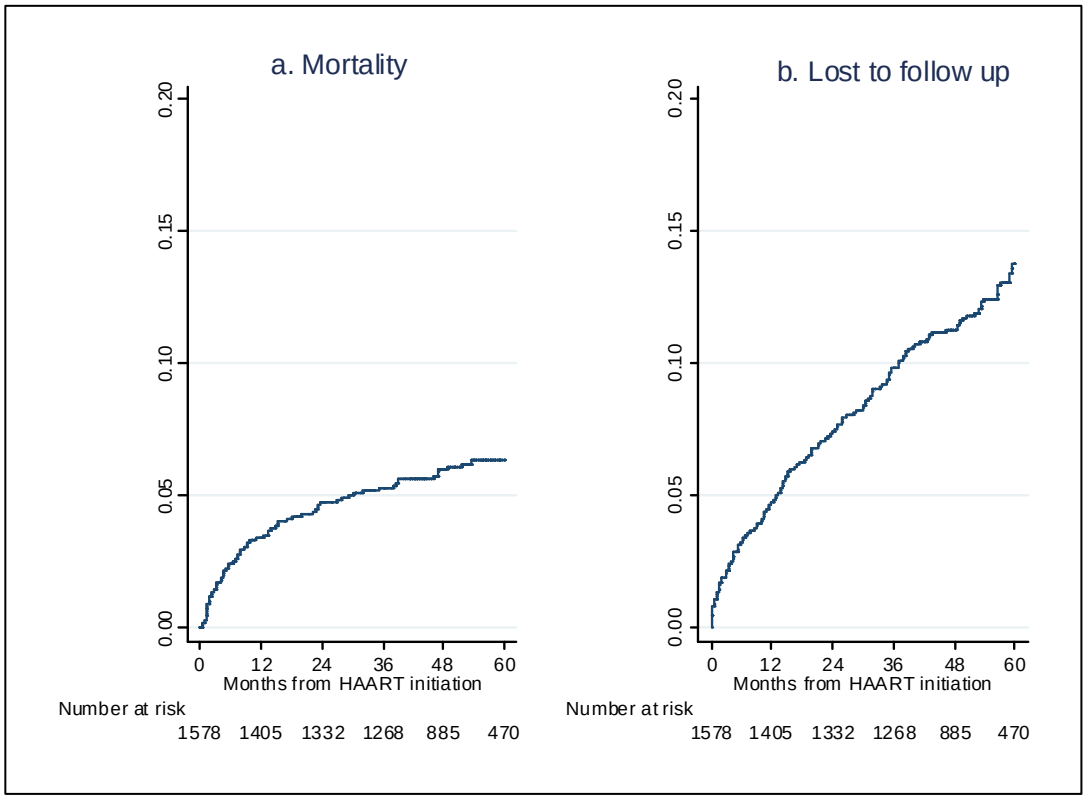
Notes

* Either an AIDS defining event (Category C of CDC classification) or an infection that has been considered as likely or definitely related to HIV using the CoDe classification (www.cphiv.dk/CoDe).
**Others are: renal failure, lactic acidosis, anemia for Early and respiratory disease, central nervous system disease for Long term deaths. |

The frequency of different major diagnosis (see categories in table 2), was not significantly different for Early and Long-term deaths (p=0.4), nor for men and women (p=0.5).

The probability of retention (alive and on follow-up or voluntarily withdrawal) was 94.4% (93.1- 95.4) at 6 months, 92.0% (90.6 - 93.3) at 1 year and 80.8% (78.5- 82.8) at 5 years. The rate of LTFU was highest during the first 6-months of therapy at 6.7 /100 PY (95% CI, 5.1- 8.8) and declined to 2.6/100PY (95% CI, 2.2- 3.0) after 6-months (Figure 2b), but it did not reach a plateau and had its estimates an overall different shape than mortality.

Fig2b. Kaplan Meier estimates: of Mortality (left) and Loss to follow up (right).



Patients lost to follow up, when compared with all others in the cohort, were younger (29 versus 35 years), more female (88% versus 72%), with less advanced CDC stage (34 versus 43% in stage C or B). They were enrolled more in initial years (57% versus 31% in 2002-3), in observational cohort (81% versus 51%) and in smaller hospitals (32 versus 20%). Treatment was NVP-based (65% versus 52%) more than EFV-based; all $p<0.05$.

Risk factors for Early mortality

In univariate analyses using the outcome of death within 6-months of HAART initiation, baseline risk factors associated with early mortality ($p<0.2$) were gender, BMI, CDC disease stage, CD4 cell count, viral load and anemia (Table 2).

These baseline covariates, together with age, were included in the multivariate analyses and factors independently associated with early mortality were: anemia with an adjusted hazard ratio (aHR) of 3.4 (95% CI, 1.7-6.8, $p=0.001$) and low CD4 with aHR 1.5 (95% CI, 1.1- 2.1, $p=0.008$) per 50 cells decrease; while high viral load had weak association ($p=0.08$). After adjusting for these factors age and sex, BMI and CDC were no longer associated with Early mortality ($p>0.1$) (Table 2).

Table 2: Early (0-6 months) and Long-term (6-60 months) mortality rates, univariate and multivariate Cox models by baseline covariates

	Early Mortality (n = 1578)						Long-term Mortality - Model 1(n = 1453)					
	Events/ PY	Mortality (95%CI)	Crude HR (95%CI)	p value	Adjusted HR* (95% CI)	p value	Events/ PY	Mortality (95%CI)	Crude HR (95%CI)	p value	Adjusted HR** (95% CI)	p value
Baseline Characteristics												
Sex: Women	23/553	4.2 (2.8 – 6.3)	1		1		32/3859	0.8 (0.6 – 1.2)	1		1	
Men	14/195	7.2 (4.2 – 12.1)	1.7 (0.9 – 3.4)	0.1	1.5 (0.7 – 3.1)	0.3	20/1327	1.5 (1.0 – 2.3)	1.8 (1.0 – 3.1)	0.05	2.5 (1.3 – 4.6)	0.004
Age (per 5 year increase)			1.1 (0.9 – 1.3)	0.4	1.0 (0.8 – 1.3)	0.8			1.1 (0.9 – 1.3)	0.3	1.2 (1.0 – 1.4)	0.1
BMI (kg/cm^2) > 18.5	22/565	3.9 (2.6 – 5.9)	1		*1*		30/3933	0.8 (0.5 – 1.1)	1		1	
≤18.5	15/175	8.6 (5.2 – 14.2)	2.2 (1.1 – 4.2)	0.02	*1.2 (0.6 – 2.4))*	*0.6*	20/1193	1.7 (1.1 – 2.6)	2.1 (1.2 – 3.8)	0.008	1.8 (1.0 – 3.2)	0.06
CDC stage A	9/414	2.2 (1.1 – 4.2)	1	1	1		20/2869	0.7 (0.4 – 1.1)	1			
B or C	28/299	9.4 (6.5 – 13.5)	4.2 (2.0 – 9.0)	<0.001	1.9 (0.8 – 4.2)	0.1	28/2047	1.4 (0.9 – 2.0)	1.9 (1.1 – 3.5)	0.02	*1.4 (0.8 – 2.5)*	*0.3*
CD4 (per 50 cell decrease)			2.0 (1.5 – 2.7)	<0.001	1.5 (1.1 – 2.1)	0.008			1.2 (1.0 – 1.5)	0.004	*1.1 (0.9 – 1.3)*	*0.6*
CD4> 50	18/574	3.1 (2.0 – 5.0)					31/3969	0.8 (0.5 – 1.1)				
CD4≤50	18/163	11.0 (7.0 – 17.5)					21/1129	1.9 (1.2 – 2.9)				
Viral load (per log increase)			2.7 (1.6 – 4.4)	<0.001	1.7 (0.9 – 3.0)	0.08			1.5 (1.0 – 2.3)	0.05	*1.3 (0.9 – 2.0)*	*0.2*
Anemia^ : No	14/597	2.3 (1.4 – 4.0)	1		1		33/4149	0.8 (0.6 – 1.1)	1		1	
Yes	17/120	14.1 (8.9 – 22.7)	5.2 (2.7 – 10.1)	<0.001	3.4 (1.7 – 6.8)	0.001	15/819	1.8 (1.1 – 3.0)	2.3 (1.2 – 4.2)	0.008	1.9 (1.0 – 3.6)	0.05
Year of initiation: 2006-8	11/293	3.7 (2.1 – 6.8)	1		1		10/1893	0.5 (0.3 – 1.0)	1		1	
2004-5	16/202	7.9 (4.9 – 12.9)	2.1 (1.0 – 4.5)		*1.2 (0.5- 2.8)*	*0.3*	14/1504	0.9 (0.6 – 1.6)	2.0 (0.9 – 4.5)		2.2 (1.0 – 5.1)	
2002-3	10/253	3.9 (2.1 – 7.3)	1.0 (0.4 – 2.5)	0.09	*0.6 (0.2- 1.6))*		28/1790	1.6 (1.1 – 2.3)	3.3 (1.6 – 6.9)	0.004	4.9 (2.2 – 10.8)	0.0003
Initial treatment: NVP based	20/402	5.0 (3.2 – 7.7)	1				31/2857	1.1 (0.8 – 1.5)	1		*1*	
EFV based	16/285	5.6 (3.4 – 9.1)	1.1 (0.6 – 2.2)				12/1898	0.6 (0.4 – 1.1)	0.6 (0.3 – 1.1)	0.01	*0.7 (0.3- 1.5)*	
PI based	1/61	1.6 (0.2 – 11.7)	0.3 (0.0 – 2.5)	0.5			9/430	2.1 (1.1 – 4.0)	2.0 (0.9 – 4.1)		*1.5 (0.7 – 3.3)*	*0.3*
FU# in: Monitoring trial	15/345	4.3 (2.6 – 7.2)	1				17/2331	0.7 (0.5 – 1.2)	1		*1*	
Observational study	22/403	5.5 (3.6 – 8.3)	1.3 (0.6 – 2.4)	0.5			35/2855	1.2 (0.9 – 1.7)	1.8 (1.0 – 3.3)	0.04	*0.5 (0.2 – 1.4)*	*0.2*
Hospital size§ : Large	28/589	4.8 (3.3 – 6.9)	1				38/4148	0.9 (0.7 – 1.3)	1			
Small	9/160	5.6 (2.9 – 10.8)	1.2 (0.6 – 2.5)	0.7			14/1038	1.3 (0.8 – 2.3)	1.5 (0.8 – 2.7)	0.2		

*Adjusted for sex, age, CDC stage, anemia, CD4 cells and viral load at baseline.
** Adjusted for sex, age, BMI, anemia and year of starting HAART **(MODEL 1)** ^Anemia = hemoglobin ≤10 g/dL, §Large ≥ 35 patients on HAART in this cohort °FU= follow-u p

The use of CD4 as continuous variable was preferred to CD4 in 4 categories. Using CD4>200 cells/mm^3 as referral category, other categories (100-200, 50-100 and <50) showed higher HR for Early mortality in univariate analysis and a trend in multivariate analysis (but p>0.05 once adjusted for other baseline variables).

Long-term mortality

At 6 months 1,453 (92%) patients remained on follow-up. Median CD4 at 6 months was 245 (IQR 168-334) cells/mm^3, median CD4 change from baseline was 114 (IQR 62-178) cells/mm^3; 1316 (92%) patients had viral load ≤1000 copies/mL, 92 (6%) had anemia and 235 (16%) had low BMI (Table 1).

Long term mortality rates were highest in patients with the following characteristics at 6 months: viral load>1000 copies, 3.1 deaths per 100PY (95%CI 1.6 – 5.7); CD4 change from baseline ≤50cells/mm^3, 2.5 per 100PY (95%CI 1.6 – 3.7); and persistent anemia, 3.2 per 100PY (95%CI 1.7 – 6.0) (Table 3).

Table 3: Long-term mortality rates, univariate and multivariate Cox models by covariates at 6 months – Model 2 (*n=1453).*

Model 2	Events/PY	Mortality rate (95%CI)	Crude HR (95%CI)	p value	Adjusted HR* (95% CI)	p value
Characteristics at 6 months						
Viral suppression: ≤1,000copies/mL	39/4780	0.8 (0.6 – 1.1)	1		1	
>1,000	10/327	3.1 (1.6 – 5.7)	3.5 (1.7 – 7.0)	<0.001	2.9 (1.3 – 6.1)	0.006
CD4 increase ≥50	24/4040	0.6 (0.4 – 0.9)	3.5 (2.0 – 6.1)	<0.001	1	
<50	23/937	2.5 (1.6 – 3.7)			2.8 (1.5 – 5.2)	0.001
BMI > 18.5	35/4336	0.8 (0.6 – 1.1)	1		*1*	
≤ 18.5	16/818	2.0 (1.2 – 3.2)	2.5 (1.4 – 4.4)	0.003	*1.7 (0.9 – 3.2)***	*0.09*
Anemia:						
Never had	28/4007	0.7 (0.5 – 1.0)	1		1	
Recovered	8/620	1.3 (0.7 – 2.6)	1.6 (0.8 – 3.6)	<0.0001	1.6 (0.7 – 3.5)	0.003
Persistent or new cases	10/308	3.2 (1.7 – 6.0)	4.9 (2.5 – 9.5)		3.6 (1.7 – 7.7)	

* Adjusted for: sex, age, BMI, year of enrollment at baseline, viral load, CD4 increase and anemia at 6 months
** Adjusted for: sex, age, year of enrollment at baseline, viral load, CD4 increase and anemia at 6 months

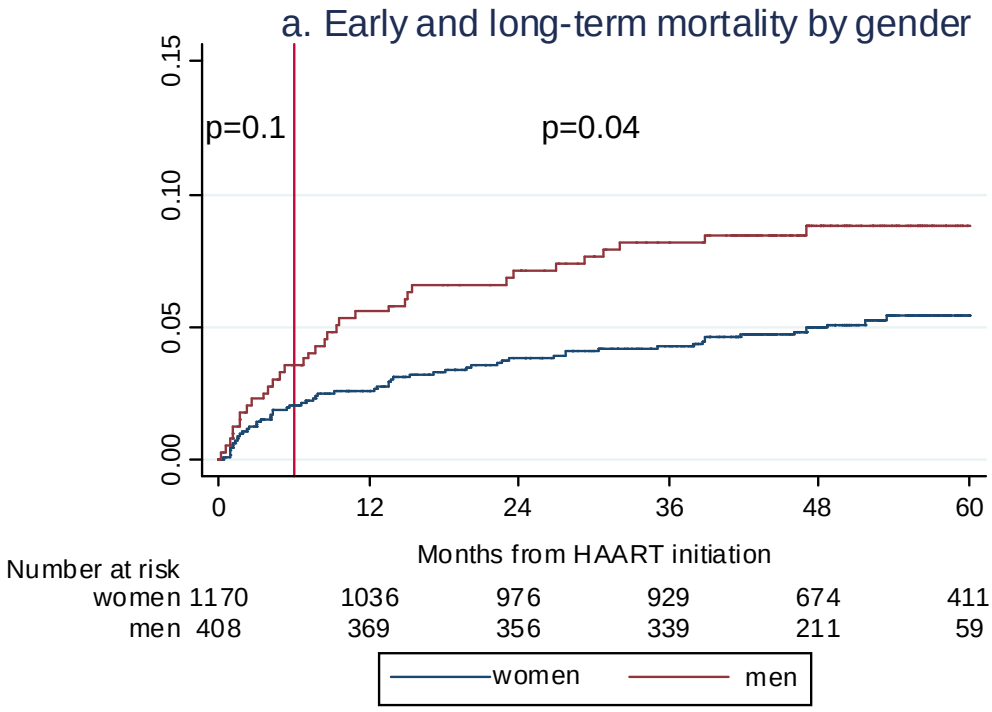
Model 1: only baseline variables

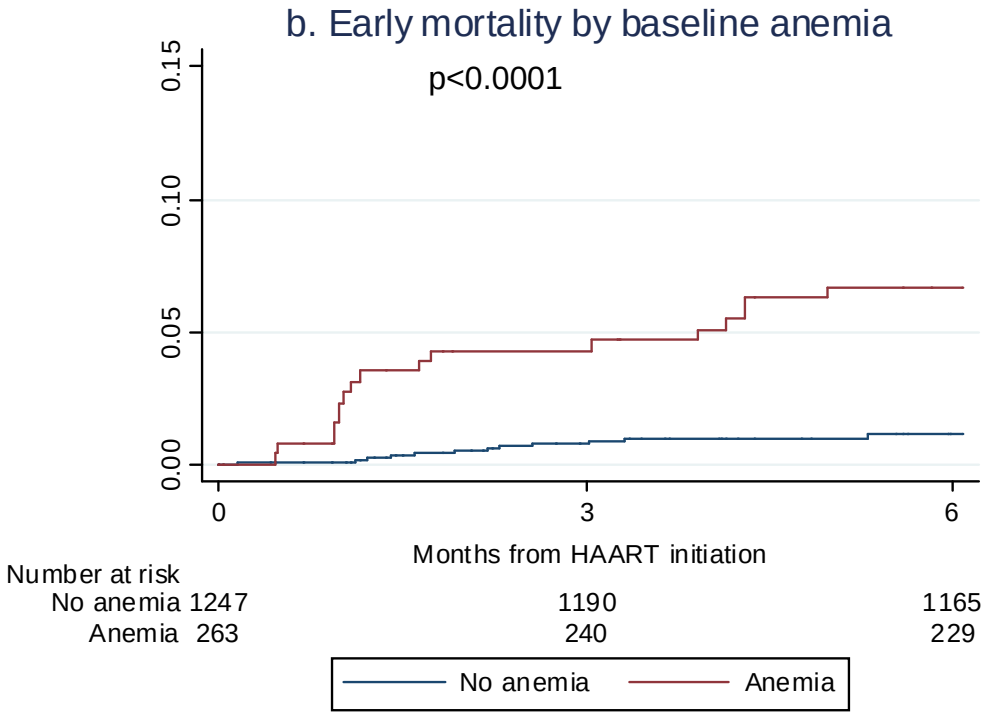
In univariate analysis baseline low BMI, CDC stage B/C, low CD4, anemia, early year or enrollment, treatment and type of follow up were associated ($p<0.05$) with long term mortality (Table 2). In the multivariate analysis, adjusting for sex and age, factors independently associated with long term mortality were: male gender (aHR 2.5, 95%CI 1.3- 4.6), early year of enrollment (aHR 4.9; 95%CI 2.2 – 10.8 for 2002-3 as compared to what >2005); and anemia (aHR 1.9; 95%CI 1.0- 3.6) and low BMI (aHR 1.8; 95%CI 1.0- 3.1) at baseline with weaker association (Table 2).

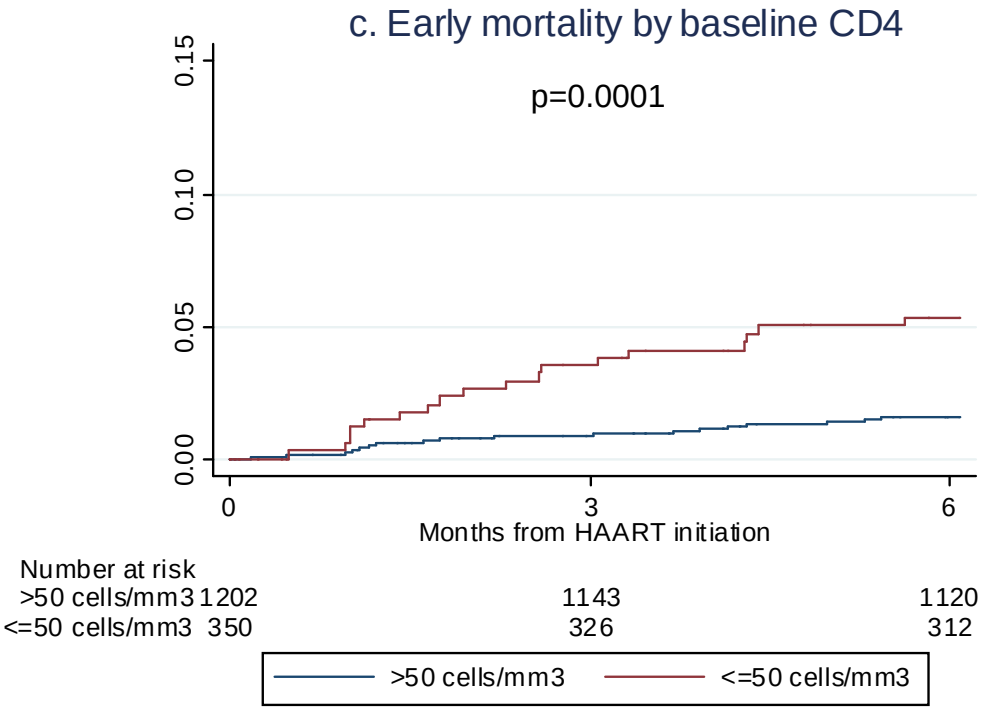
Model 2: variables at baseline and at 6-months

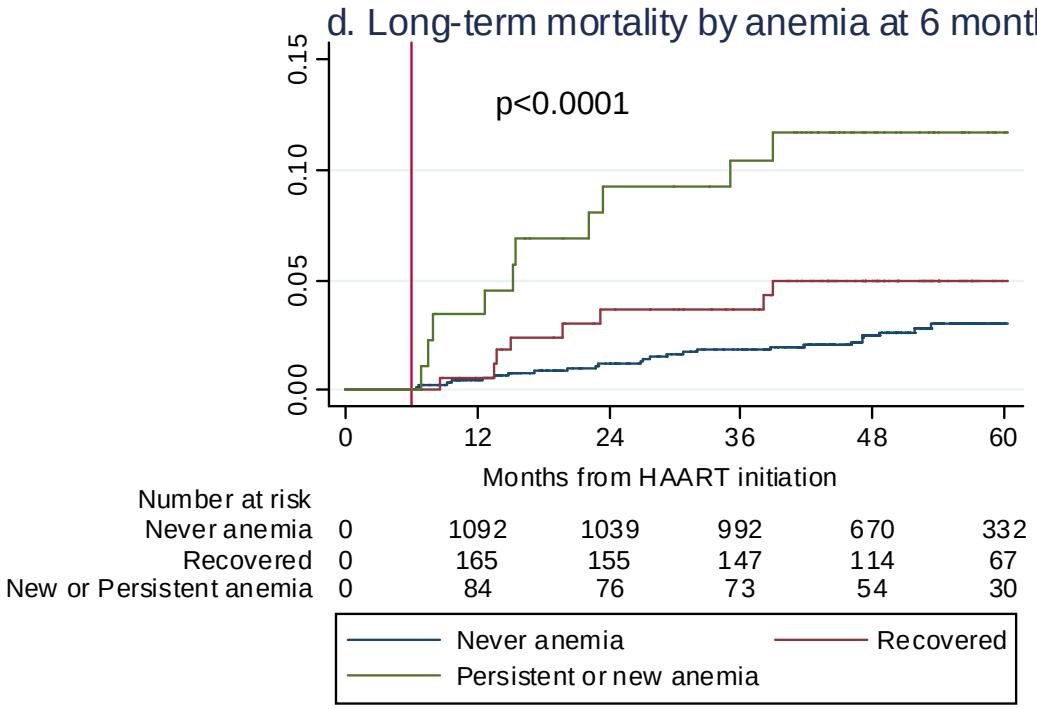
In univariate analysis, all covariates at 6 months were associated ($p<0.05$) with long-term mortality (Table 3, Figure 3). In multivariate analysis, adjusting for sex, age and associated baseline variables, factors independently associated with long term mortality were: viral load>1000 copies/mL (aHR 2.9 95%CI 1.3- 6.1); CD4 increase $\leq$50 cells/mm^3 at 6 months (aHR 2.8 95%CI 1.5- 5.2) and anemia (*persistent or new* aHR 3.6 95%CI 1.7 – 7.7) (Table 3). Results were similar if using CD4$\leq$100 cells/mm^3 at 6-months (aHR2.5 95%CI 1.2 – 5.3) instead of CD4 increase.

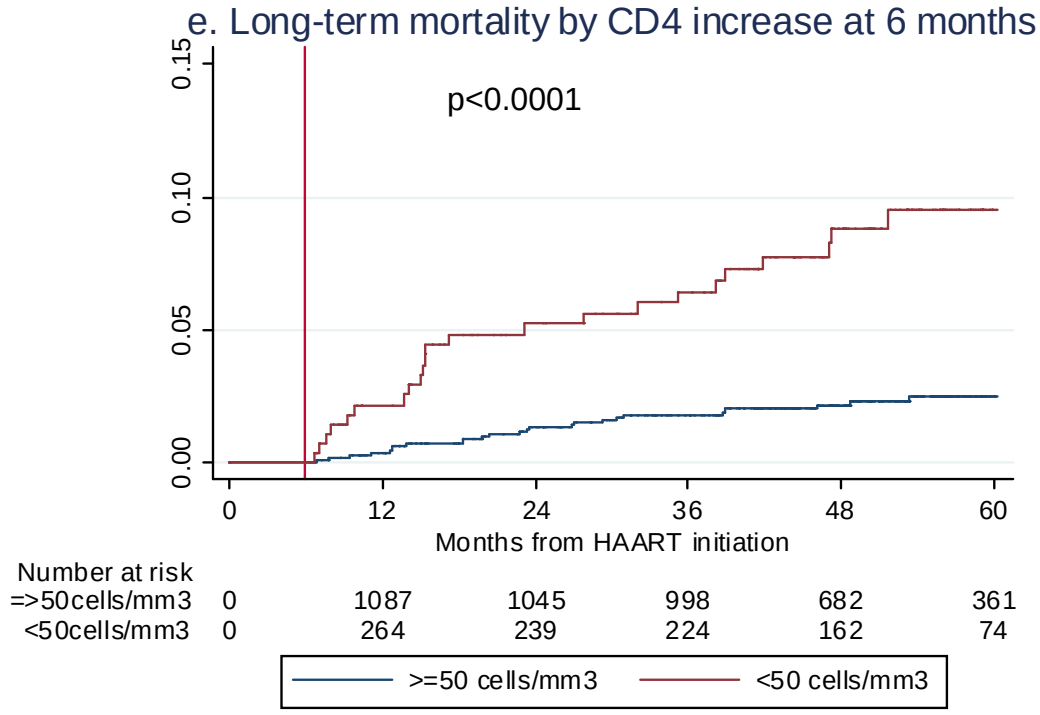
Figure 3: Kaplan Meier estimates of Early mortality (a, b, c) by characteristics at baseline and of Long term mortality (a, d-f) by characteristics at 6 months. (p for log rank test reported)

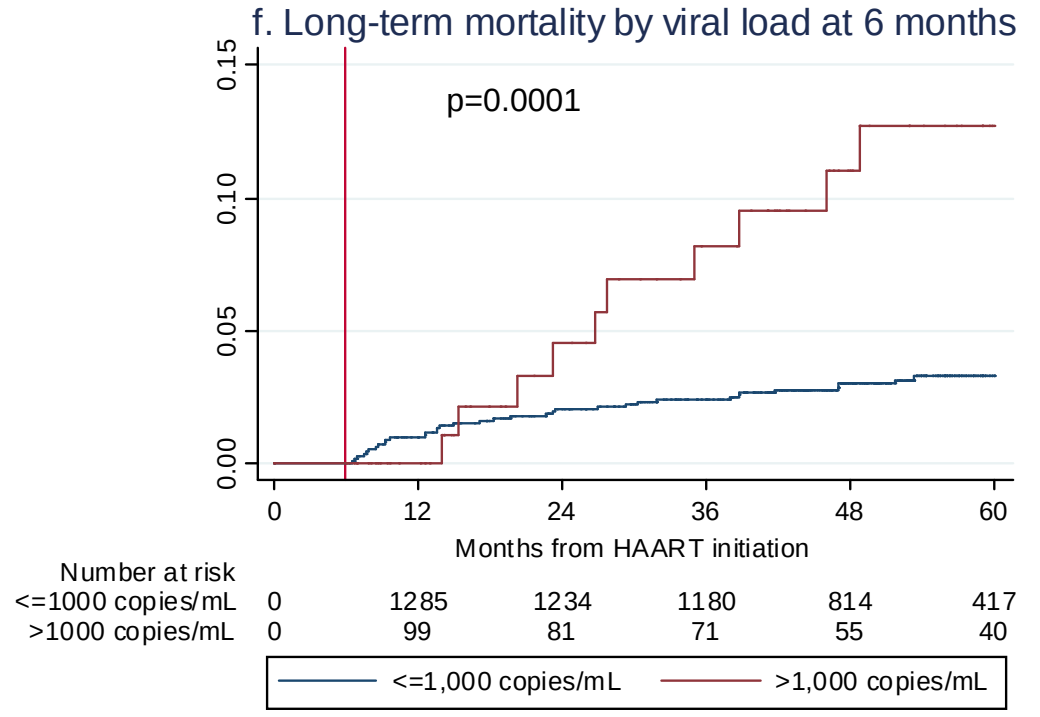





c. Early mortality by baseline CD4
p=0.0001
0.15
0.10
0.05
0.00
0
3
6
Months from HAART initiation
Number at risk
>50 cells/mm3 1202 1143 1120
<=50 cells/mm3 350 326 312
>50 cells/mm3
<=50 cells/mm3


d. Long-term mortality by anemia at 6 months
p<0.0001
0.15
0.10
0.05
0.00
0
12
24
36
48
60
Months from HAART initiation
Number at risk
Never anemia 0 1092 1039 992 670 332
Recovered 0 165 155 147 114 67
New or Persistent anemia 0 84 76 73 54 30
Never anemia
Recovered
Persistent or new anemia





Note : An alternative definition of unsuppressed 6 months viral load (>1,000 copies OR not done at 6 months) has been tested. The graph of estimates corresponding to this definition are presented in Appendix 1.

All other variables at baseline and at 6 months were not significantly associated with Long-term mortality in multivariate analysis.

Baseline CD4 were not associated with mortality once controlled for other baseline characteristics. CD4 at baseline was used as a continuous variable (by 50 cells decrease). Using it as categorical with in 4 grades (>200, 100-200, 50-100 and ≤50) or 2 (with cut off either 100 or 50) did not change the significance level and they were excluded from the final model 1 as associated p>0.2.

Interestingly, both increase in CD4<50 cells/mm^3 and absolute CD4≤100 cells/mm^3 at 6 months, were associated with long-term mortality. More in detail, these 2 variables distribution is as follow:

CD4 at baseline (cells/mm^3)	**CD4 increase <50 cells/mm^3**	**CD4 ≤ 100 cells/mm^3 at 6 months**
< 50	55 (19)	97 (82)
50 – 100	35 (12)	12 (10)
100-200	91 (32)	6(5)
> 200	103 (36)	1 (1)
unknown	--	3 (3)
Total	284 (100)	119 (100)

A total of 119 (8%) patients had ≤100 CD4 and 284 (20%) had <50cells increase at 6 months.

Subjects with CD4<100 are mostly patients who had low CD4 at baseline, while CD4 change <50 are patients with any baseline CD4.

Subgroup analysis of survival at 3 years

As all patients have been enrolled in the cohort at least 3 years before the censoring date for the analysis, we performed a subgroup analysis of risk factors for mortality at 3 years.

Variable considered; number of patients and analysis methods were the same applied for the 5 years analysis.

Results of analysis at 3 years from starting HAART:

Among the 1453 patients still alive and on follow-up at 6 months, there are 41 deaths occurring between 6 and 36 months, in a total of 3,379PY of follow-up (mortality rate 1.2/10PY 95%CI 0.9 – 1.6).

In a model using only baseline variables, predictors of mortality between 6-36 months were: male gender and earlier calendar year, controlling for age, anemia and BMI:

Multivariate analysis for mortality at 3 years, baseline variables only:

Characteristics at baseline	Adjusted HR* (95% CI)	p value/ test parm
Sex : female	1	
male	3.2 (1.6 – 6.3)	0.001
Age (for 5 years)	1.2 (1.0 – 1.5)	0.1
Anemia No	1	
Yes	2.0 (1.0 – 4.1)	0.06
BMI > 18.5	1	
≤ 18.5	1.9 (1.0 – 3.7)	0.05
Calendar year :		
>2005	1	
2004-5	2.8 (1.1 – 7.4)	0.0002
2002-3	6.9 (2.8 – 17.3)	

*adjusted for all other variables in the table

In a second model, including variables at 6 months and the baseline variables, predictors were still male gender (aHR 3.4, p<0.001) and earlier calendar year (aHR 2.3 and 6.4, p=0.0003), and anemia at 6 months (aHR 4.5, p<0.001), change in CD4 (aHR 2.2, p=0.04), viral load at 6 months (aHR 2.5, p=0.04) and BMI at 6 months, controlling for age.

Multivariate analysis for mortality at 3 years; baseline and 6 months variables:

Characteristics at baseline	Adjusted HR* (95% CI)	p value
Sex : female	1	
male	3.9 (1.9 – 8.1)	<0.001
Age (for 5 years)	1.2 (1.0 – 1.5)	0.05
Calendar year :		
>2005	1	
2004-5	2.3 (0.9 – 6.2)	0.0003
2002-3	6.4 (2.5 – 16.5)	
Viral suppression	1	
	2.5 (1.0 – 6.2)	0.4
Anemia No	1	
Yes	4.5 (2.1 – 9.8)	<0.001
BMI >18.5	1	
< 18.5	2.0 (1.0 – 3.8)	0.05
CD4 increase ≥50	1	
<50	2.2 (1.0 – 4.5)	0.4

* adjusted for all other variables in the table

Conclusion of analysis at 3 years:

As for analysis at 5 years, baseline characteristics predictive mortality after 6 months (and before 36 months) are male gender and early calendar year. Among the variables measured at 6 months: low change in CD4 (<50 cells/mm^3), viral load at 6 months > 1,000 copies/mL and anemia at 6 months are predictive of later mortality.

Predictive role of sex in long-term mortality:

In our analysis male sex was associated with higher risk of long term mortality. When performing an analysis stratified for gender, the final results were similar to the not stratified analysis. Predictors of long term mortality being earlier calendar year (p= 0.0007), low change in CD4, unsuppressed VL at 6 months, and anemia at 6 months, controlled for age and BMI at 6 months.

Risk factors for men and women were also analyzed separately. The cohort of men included 380 patients, and accounted for 20 deaths in the long-term period. Anemia at 6 months (aHR 7.5, p=0.004), early calendar year (aHR2.0 for 2004-5 and aHR5.0 for 2002-3, p=0.03), older age (aHR1.1, p=0.02 for 5 years increase) controlling for baseline CDC (aHR 3.3, p=0.1), were the risk factors for long-term mortality.

For the cohort of women (n=1,073, 32 deaths), the risk factors were low CD4 at 6 months, (aHR6.1, p<0.001), unsuppressed VL at 6 months (aHR 4.3, p<0.001) and anemia at baseline (aRH 2.4, p=0.03), once controlled for age.

Also, men and women had no difference in distribution of Early vs Long-term deaths (42% early deaths for women and 41% for men).

Determinants of anemia at 6 months

To identify the baseline characteristic associated with anemia at 6 months univariate and multivariate logistic regressions have been performed using the baseline variables considered for the previous Cox models.

In univariate analysis female gender, BMI, CDC stage B or C, baseline CD4, baseline anemia, starting a ZDV containing regimen, early calendar year of enrollment, being in cohort(vs monitoring clinical trial), being followed in a smaller hospital were associated with having anemia ta 6 months (defined as hemoglobin < 10g/dL). All the above has been introduced in a multivariate analysis with forward selection and kept in the model if associated p<0.1.

The baseline variables associated to anemia at 6 months were: anemia at baseline (aOR 9.8, 95%CI 8.5- 11.4), female gender (aOR 4.3, 95%CI 3.3- 5.5), ZDV in initial treatment (aOR 3.1, 95%CI 2.7 – 3.6), and earlier calendar year (aOR 1.2 95%CI 1.1 – 1.5), all p<0.05.

Results of multivariate logistic regression (dependent variable: anemia at 6 months):

Baseline characteristics	OR* (95%CI)
Anemia (hemoglobin <10g/dL)	9.8 (8.5 - 11.4)
Sex: female	4.3 (3.3 - 5.5)
ZDV-based initial treatment[§]	3.1 (2.7 – 3.6)
Earlier calendar year (<2006)	1.2 (1.1 – 1.5)

*adjusted for cd4 baseline
[§] compared with all treatments not-ZDV based

Discussion of analysis and results in light of current literature:

In our study of previously untreated adults receiving HAART, the mortality rate was highest during the first 6-months of therapy at 4.9 deaths per 100PY, comparable to reports from Africa and Asia. However, after 6-months of HAART the mortality fell to 1.0 deaths per 100PY, similar to rates of 1.1-2.6 deaths per 100PY reported by US and European cohorts (3, 17-19).

The probability of survival at 1-year of HAART in our cohort was 96.6%, this is higher than previous reports from resource limited settings: sub-Saharan Africa (74-92%) (4), Latin America (87-97%) (20) and Asia (Thailand 89%, Cambodia 87%) (21, 22). The higher survival rate observed in our study may be due to patients starting therapy at less advanced disease stage: 25% in CDC stage C versus more than 50% in sub-Saharan African studies; median baseline CD4 was 124 versus 41 CD4 cells/ml in previous report from Thailand (21, 23, 24). This maybe partly explained by the large proportion of women in our cohort (74%), many of whom were referred from PMTCT programs and received continued monitoring to allow initiation of therapy prior to advanced disease progression.

Our high survival rate at 5 years (93.7%) is comparable with one clinical trial in Uganda, the DART trial comparing clinical versus laboratory monitoring on ART. It represents one of the few large cohorts on treatment for up to 5-years, and they reported a survival rate of 88% at 5-years, although patients had lower CD4 (median 86cells/mL) and more advanced disease stage at initiation of therapy (80% in WHO stage III or IV) as compared to our cohort (25).

Early mortality:

As reported in other studies, early mortality was strongly associated with low baseline CD4 count and anemia (2, 4, 23, 26-29). In contrast baseline viral load and clinical stage, which were reported as risk factors in other studies (3, 4, 21) had weak association in our study, probably for

the effect of other variables included in the model, as anemia. Most of the early deaths were attributed to infections related to immunodepression.

Our study, as others showing high early mortality [*4,19,21*] and even higher mortality in the pretreatment period (24), highlights the need for strategies to treat patient earlier. Expanding access to rapid diagnosis, with provider initiate testing in different healthcare settings, and starting treatment at earlier disease stage, are recommended by WHO guidelines [29] (30). Continued follow up of newly diagnosed patients, including peer support, home visits and contact tracing, could avert LTFU during screening and ensure timely initiation of therapy. In addition, efficient screening and treatment of opportunistic infections, in particular tuberculosis, is needed in patients starting treatment in advanced disease stage (5), in order to reduce early mortality.

Late mortality:

Mortality after 6 months declined but remained not negligible, accounting for 58% of all deaths in this cohort. The mortality rate of 1.0 per 100PY is higher than in the Thai general population, where adult mortality rate in age range 30-34[1] was 0.53/100 in 2008: 0.20 for female and 0.51 for men (WHO Global Health Observatory http://apps.who.int/ghodata/?vid=720).

When including variables at 6-months of therapy, strong predictors of long term mortality were CD4 increase of <50 cells/mm^3 from baseline, viral load>1000 copies/mL and persistent anemia at 6-months. After adjusting for these factors the only baseline variables predictive of long-term mortality were male gender and early year of initiation.

These findings are applicable only to the subgroup of the population who survived and remained on follow up at 6 months. They are consistent with previous reports showing response to treatment at 4 or 6 months was important in predicting late mortality (12, 17, 24, 27). In particular unsuppressed viral load during the first months of therapy has been shown to be a strong predictor

[1] Age group chosen for comparison as median age of cohort is 33 years.

of subsequent mortality (12, 17, 27) as well as CD4 cell count at 6 months or CD4 change from baseline to 6-months (12, 17, 24).

Baseline CD4 was not associated with Late mortality, as in studies utilizing updated CD4 (12, 24). In a study of North American and European cohorts (31) CD4 at baseline was still prognostic after years of follow-up but only for the lowest category (<25cells/mL).

The predictive role of baseline or updated values of hemoglobin for Late deaths has been demonstrated in Africa (26, 27) and Europe (32). In our study, patients with anemia at baseline were at high risk of Early mortality; and patients with persistent anemia at 6 month had high risk of subsequent death (aHR3.6, 95%CI 1.7- 7.7). Anemia in patients with HIV could be due to different causes: OI and other infections, HIV infection itself, nutrients deficiency and side effects of therapy – either antiretroviral or antimicrobial (33). After starting HAART the percentage of patients with anemia progressively decreases (34), but current anemia remains an independent risk of disease progression and death (35). In our cohort, stronger association with anemia at 6 months were, aside from anemia at baseline, female gender, starting a ZDV-based treatment and earlier calendar year. Even if the patho-physiological role of anemia is not yet completely understood, its correction may improve survival.

Effect of gender on survival is still debated as results to date are discordant (21, 24, 36-41). In a review on HAART and gender (42), virological response, adherence and adverse drugs reactions differed in men and women, with an overall better survival for women. In our cohort men had higher Late but not Early mortality rates. Men were older (mean age 37 vs 33 years) and at more advanced disease stage (CDC stage B or C 59 vs 36%), but had less anemia at baseline (11 vs 20%) and at 6 months (2 vs 7%) and were more likely to achieve viral suppression 6-months (27 vs 13%); all p<0.001. Main causes of death did not differ between women and men. After adjusting for clinical and virologic variables, gender remained associated with higher mortality after 6 months of therapy: men had mortality of 1.5 deaths/100PY (95%CI 1.0-2.3) versus 0.8/100PY (95%CI 0.6-1.2) of women. This maybe due to unmeasured factors, including co-infections, long-term

adherence, socio-behavioral factors or mode of transmission. Furthermore, male mortality was higher in the Thai general population before the AIDS epidemic (43).

Calendar year was associated with mortality as observed in other studies (44, 45), this most likely reflects improvements in standard of care and medical team experience over time, better regimens available, improved treatment monitoring and improved health status of patients at initiation of therapy. However there was no effect of calendar time on early mortality suggesting it may continue to be driven by the significant proportion of patients who were severely immuncompromised and at high risk of early death.

Strengths and limitations

This is one of the few cohorts of ART-naïve patients receiving HAART with long-term prospective follow-up to 5 years, with high retention rates and few missing data. In addition, all missing data were imputed using MICE.

Treatment and care were provided to patients in 43 public hospitals throughout the country, the hospitals ranged from small peri-urban hospitals to large tertiary university hospitals. The cohort received care by health care teams who also implement the national treatment program, while some patients were enrolled in a nested trial, the large majority received care that was close to standard of care and is considered to be generalizable to comparable settings.

Losses to follow-up could cause underestimation of mortality. In some African cohorts tracing vital status of patients lost to follow-up changed dramatically the estimated mortality (45-47). In our case we do not think that the majority of patients lost to follow up represent unseen deaths, as free access to HAART was rapidly scaled up throughout the country and patients leaving the program could have accessed treatment elsewhere. Also, the risk factors associated with mortality were different from those associated with LTFU, except for earlier calendar year of enrollment. In brief, younger age and higher BMI at baseline were strongly associated with LTFU, after controlling for

gender, CDC stage, CD4, initial regimen and study site size. Anemia and high viral load at baseline were not associated in the univariate or multivariate analysis. In addition, because in our study, both LTFU rate (12%) and mortality rate (6%) at 5-years were relatively low, even if patients lost had higher probability of death than patients on follow-up, survival estimated would not change substantially.

The analysis did not include some potential risk factors such as adherence or socio-economic status (48, 49), although viral load response at 6-months was considered a proxy of early adherence. Also, while we used values updated at 6 months, it could be useful in further analysis to explore the role of more current updates (for example at 12 or 24 months) (50). We did not include exposure to single-dose NVP for PMTCT as a risk factor due to missing data for a large number of women. Studies in Thailand and Africa have reported poorer virologic response among women exposed to SD-NVP initiating NNRTI first line regimens, however no survival difference was observed (51-53).

It was not possible in this study to ascertain if treatment received initially or at 6 months was a predictor of mortality as treatment was not randomized and available regimens changed over time and were strongly correlated with calendar year.

Conclusions

In conclusion, high survival rates were achieved and sustained at up to five years of antiretroviral therapy in a lower-middle country. Compared to extremely high mortality rates in patients without therapy (54), this reflects the tremendous results achievable with potent and affordable antiretroviral treatment in resource limited settings. Nevertheless, risk of death once on HAART is still not negligible and response to treatment and patients' status at 6 months are important predictors for later deaths. To decrease early and late mortality possible strategies would therefore be: starting treatment earlier, increasing adherence since the first months, promptly diagnose and manage possible causes of anemia, including underlying conditions and treatment toxicity.

Note: references at the end of the three studies section

End stage liver diseases in chronic HCV infection.

Predictors of end stage liver disease (ESLD) in patients with HCV co-infected with HIV

This research work has been carried out by the candidate in collaboration with the Research Institute of McGill University Health Center (MUCH), at Chest Institute, McGill University; Montreal, Canada.

The work consisted in the first part of the development of a model able to predict who, among the patients followed for HCV infection, would have higher risk of development of liver cirrhosis or other clinical manifestations of the end stage liver diseases (ESLD). The part of the project here presented focused on selection of the variables useful to develop the model. Results of this part have been presented in an international workshops and conferences (13th International Workshop on HIV Observational Databases. Lisbon, Portugal- March 2009 and 18th Annual Conference on HIV/AIDS Research. Vancouver, Canada-April 2009). As the cohort is still enrolling participants, an update analysis performed on higher number of patients and longer follow-up time is ongoing. Pending completion of the final model with use of updated data, a full paper will be submitted to a peer-reviewed journal.

The patients' characteristics utilized to build the model of patients' with HIV and HCV infection, at the time in which the join the monitoring cohort.

Introduction

ESLD is a clinical entity given by presence of one of more major liver damages, as for example decompensated cirrhosis or bleeding esophageal varices

Chronic HCV infection of the liver results often in one or more of these complications and ESLD is even more common in patients with HCV co-infected with HIV.

Having a scoring system, to predict individual risk on the base of clinical and biological markers, would be useful to target management and prevention of ESLD in the population wit co-infection.

Objective of the study was to explore which patient's characteristics could be included in a model to predict the likelihood of ESLD in chronically co-infected patients.

Methods:

Study and population:

Data were analyzed from participants in a Canadian, multi-site cohort of HIV-HCV co-infected persons (the Canadian Co-infection Cohort Study, established since 2002). Participants are followed in 16 centers in Canada. Every 6 months participants completed questionnaires on: socio-demographic, drug and alcohol use, risk behaviors, smoking habits, quality of life and clinical care, HIV and HCV treatment. A blood sample was collected every 6 months for viral, biochemical and immunologic assessments.

Detailed description of the multi-site cohort is available at http://www.cocostudy.ca/index.html; an extract of description is presented in the appendix (see appendix 2A)

Data were sent for data management and analysis at the Chest Institute, Montreal. For this analysis were utilized data collected up to 30 September 2008.

Statistical analysis:

Descriptive statistics, univariate and multivariate logistic regression models were used to evaluate risk factors associated with developing a first diagnosis of ESLD.

ESLD was defined as presence of any among decompensated cirrhosis, hepatic encephalopathy, bleeding esophageal varices, ascites, spontaneous bacterial peritonitis, hepatoma, and death attributable to liver cirrhosis.

Possible risk factors considered were based on previous literature on ESLD and HCV infection. A summary of the results from the literature review on predictors of cirrhosis and ESLD are reported in the table below.

Data were analyzed with SAS 9.2 software program (SAS Institute Inc, USA).

Results from the review of previous studies on predictors of cirrhosis and ESLD:

AUTHOR	Patients (number and follow-up) and study	OUTCOME	Risk factors
Studies on predictors of mortality once ESLD has occurred			
Child-Turcotte-Pugh 1973	38 pts with bleeding oesophageal varices	Death	Bilirubine, albumine, PT, ascites, encephalopathy
Malinchoc M, 2000, US (MELD)	231; median (range) follow up 1.1 years (0.1 to 3.8). Prospective cohort	Death within 3 months after TIPS (110 died)	Creatinine, bilirubine, INR, cause of liver disease (alcoholic or cholestatic versus other)
Studies on predictors of complications once cirrhosis is known			
Sinn, 2008, Korea	1137 HCV+ (490 pts had HCV therapy) Retrospective study	Disease progression defined as increase of 2 points of CTP score, variceal bleeding, SPB, hepatic encephalopathy, death related to liver disease, HCC. Annual incidence rate:0.8% in HCV treatment, 3.7% HCV no-treatment	Pts with HCV therapy: Treatment response, PLT, APRI. Pts without HCV therapy: Age, male, diabetes, PLT, APRI.
Sharma SK, 2007, India	101 pts with newly diagnosed cirrhosis (of all etiologies). Prospective study	Large esophageal varices: 46 pts	Low platelet count, palpable spleen
Gentile, 2008 Italy	254 with chronic liver disease from HBV/HCV	Varices (small or none). Large varices found in 12.2%. 50% of [patients had score <2 (low risk for varices).	Score based on age (>50 ys), PLT <150,000; AST/ALT ratio

AUTHOR	Patients (number and follow-up) and study	OUTCOME	Risk factors
Mendes-Correa, 2008, Brazil	234, co-infected HIV/HCV. HCV infections estimated of 16.5ys duration, FU in 1996-2004	Liver fibrosis grade 3 or 4 at biopsy (found in 25% of pts)	Older age (as continuous variable), HCV genotype 3. Note: Excluded pts for incomplete records and HBV+. Excluded pts with less than 50 CD4 cells/mm^3, as not undergoing biopsy. Pts with normal ALT included.
Martin- Carbonero, 2004, 4 EU countries	914 , HIV/HCV co-infected; 1992-2002	Severe fibrosis at biopsy (F3=22%, F4=13%) in pts with high ALT	Age > 35ys, alcohol >50 g/day, CD4<500cells/mL Not associated were: gender, transmission route, duration of HCV infection, HCV genotype, HCV RNA, HAART Note : 25% admitted alcohol. Duration of HCV infection 16 ys. Excluded if HBV, normal ALT, prior HCV therapy.
Barreiro, 2006 Spain	283 coinfected HIV-HCV. Cross sectional study	Advanced liver fibrosis (F2-F4) at fibroscan	HCV genotype 3, older age, high ALT
Sanchez-Conde, 2006, Spain	256 coinfected HIV-HCV. Cross sectional	Advanced stage of fibrosis (F3-F4) at biopsy	Advanced fibrosis more common in patients with high ALT vs normal ALT
Guaraldi 2008, Italy	225 HIV+, no HCV/ HBV co-infection nor alcohol abuse	NFALD (nonalcoholic fatty liver disease) at CT : found in 83 pts	AST/ALT ratio, male sex, waist circumference, use of NNRTI
Paggi 2008 Italy	430 with confection HIV/HCV	Severe fibrosis	APRI, nodularity at the liver surface ultrasound. Note: use of an algorithm based on 2 single or sequentially combined tests. Pts with high ALT/AST.

AUTHOR	Patients (number and follow-up) and study	OUTCOME	Risk factors
Benvegnù 2001, Italy	370 pts median 76.4 months of follow up. Prospective cohorts.	HCC in cirrhosis. Total HCC :61 (16.5%) Nodular HCC: 49 (80.3%) Infiltrating HCC: 12 (19.7%)	For HCC: older age (p=0.0002; relative risk (RR) 3.1; 95% CI 1.6-5.2), longer duration (p=0.09; RR 2.6; 95% CI 1.8-3.4), and more advanced stage (p=0.002; RR 2.5; 95% CI 1.3-4.5) of cirrhosis For infiltrating form: viral etiology(B/C)
Fattovich 2002 Italy	297 pts Median FU: 6.6 years. Retrospective cohort.	HCC, decompensation and mortality	RR (95% CI) for HCC, decompensation and mortality was 1.53 (CI = 0.81-2.89), 0.59 (CI = 0.37-0.94), and 1.44 (CI = 0.85-2.46) respectively, in **HbsAg-**positive patients compared with anti-HCV-positive cirrhotic patients
Bruno 2007, Italy	69 patients with compensated cirrhosis among 392 HIV + or co-infected. Follow up 6 years, between 1996-2004. Retrospective cohort with cases and controls.	Time to decompensation and mortality from liver related causes. 22/69 died (all liver disease related)	HAART after the first event of decompensation. Gender, age CD4, albumin, HCV genotype: no association. Note: None received HCV therapy. Possibly HAART is proxy for other conditions.
Kumar 2007 India	Case-control study: 213 HCC cases and 254 controls	HCC	HBV; HCV in cirrhotic patients

References for table above

Barreiro, P., L. Martin-Carbonero, et al. (2006). "Predictors of liver fibrosis in HIV-infected patients with chronic hepatitis C virus (HCV) infection: assessment using transient elastometry and the role of HCV genotype 3." Clin Infect Dis **42**(7): 1032-9.

Benvegnu, L., F. Noventa, et al. (2001). "Evidence for an association between the aetiology of cirrhosis and pattern of hepatocellular carcinoma development." Gut **48**(1): 110-5.

Bruno, R., P. Sacchi, et al. (2007). "Natural history of compensated viral cirrhosis in a cohort of patients with HIV infection." J Acquir Immune Defic Syndr **46**(3): 297-303.

Fattovich, G., M. Pantalena, et al. (2002). "Effect of hepatitis B and C virus infections on the natural history of compensated cirrhosis: a cohort study of 297 patients." Am J Gastroenterol **97**(11): 2886-95.

Gentile, I., C. Viola, et al. (2009). "A simple noninvasive score predicts gastroesophageal varices in patients with chronic viral hepatitis." J Clin Gastroenterol **43**(1): 81-7.

Guaraldi, G., N. Squillace, et al. (2008). "Nonalcoholic fatty liver disease in HIV-infected patients referred to a metabolic clinic: prevalence, characteristics, and predictors." Clin Infect Dis **47**(2): 250-7.

Kumar, M., R. Kumar, et al. (2007). "Risk factors analysis for hepatocellular carcinoma in patients with and without cirrhosis: a case-control study of 213 hepatocellular carcinoma patients from India." J Gastroenterol Hepatol **22**(7): 1104-11.

Malinchoc, M., P. S. Kamath, et al. (2000). "A model to predict poor survival in patients undergoing transjugular intrahepatic portosystemic shunts." Hepatology **31**(4): 864-71.

Martin-Carbonero, L., Y. Benhamou, et al. (2004). "Incidence and predictors of severe liver fibrosis in human immunodeficiency virus-infected patients with chronic hepatitis C: a European collaborative study." Clin Infect Dis **38**(1): 128-33.

Mendes-Correa, M. C., A. Widman, et al. (2008). "Incidence and predictors of severe liver fibrosis in HIV-infected patients with chronic hepatitis C in Brazil." AIDS Patient Care STDS **22**(9): 701-7.

Paggi, S., A. Colli, et al. (2008). "A non-invasive algorithm accurately predicts advanced fibrosis in hepatitis C: a comparison using histology with internal-external validation." J Hepatol **49**(4): 564-71.

Pugh, R. N., I. M. Murray-Lyon, et al. (1973). "Transection of the oesophagus for bleeding oesophageal varices." Br J Surg **60**(8): 646-9.

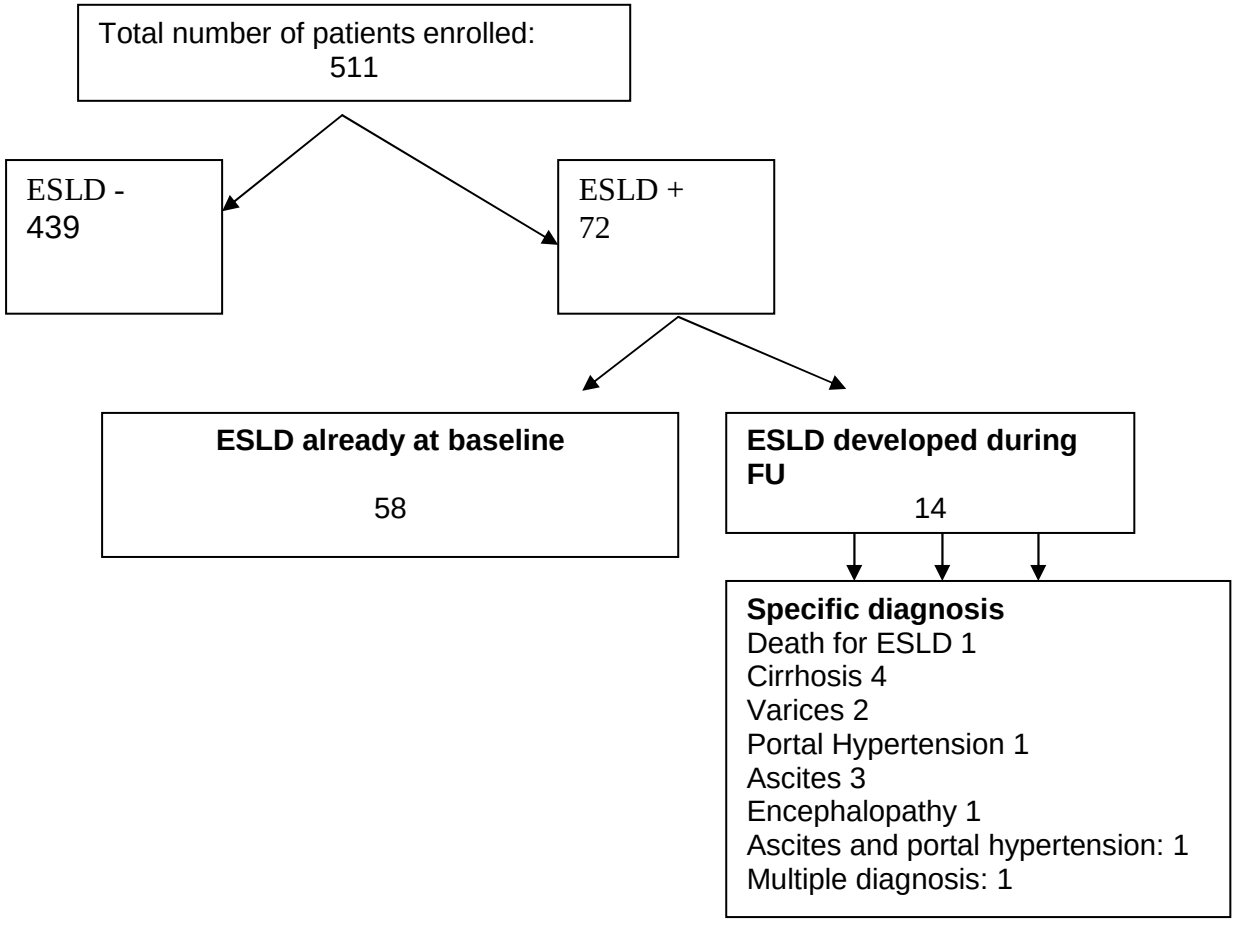
Sanchez-Conde, M., J. Berenguer, et al. (2006). "Liver biopsy findings for HIV-infected patients with chronic hepatitis C and persistently normal levels of alanine aminotransferase." Clin Infect Dis **43**(5): 640-4.

Sharma, S. K. and R. Aggarwal (2007). "Prediction of large esophageal varices in patients with cirrhosis of the liver using clinical, laboratory and imaging parameters." J Gastroenterol Hepatol **22**(11): 1909-15.

Sinn, D. H., S. W. Paik, et al. (2008). "Disease progression and the risk factor analysis for chronic hepatitis C." Liver Int **28**(10): 1363-9.

Results

A total of 511 patients were enrolled in the cohort between March 2003 and September 2008, followed for a median (IQR) of 11 (6-31) months. Fifty-eight patients (11%) already had a diagnosis of ESLD at enrolment and therefore were excluded from these analyses (See patients' flow chart below). The single most common diagnosis in patients with ESDL at entry was liver cirrhosis (35/58).



At baseline, 361 (80%) of patients were male and had a median (IQR) age of 43 (39-48) years, 75% had less than secondary school education, 60% had a monthly income <1,000$ CAN and 34% were active injecting drug users (IDU). The median (IQR) duration of HIV and HCV infections

was 19 (13-25) years and for 74% the probable route of acquisition of HCV was use of injecting drugs. (See table 1)

Fourteen patients (3%) had their first diagnosis of ESLD a median (IQR) of 12 (6-22) months after enrolment in the cohort (rate 1.98/100PY). Median baseline CD4 (IQR) was lower at 270 (176-373) in patients who subsequently developed ESLD vs. 381 (247-531) cells/ml (p=0.03) and serum creatinine (85 vs. 74 umol/L, p=0.03) and proportion with APRI> 1 (62% vs. 29%, p=0.03) were higher in the patients developing ESLD. Demographic and behavioural characteristics, CD4 nadir, proportion with HIV RNA <50 copies (55%) and on antiretroviral treatment at enrolment (79%) were not significantly different in the 2 groups nor were other hepatic, haematological or metabolic indices. A total of 62 patients (14%) had received previous HCV treatment (but only 1 of those developing ESLD). (See table 1).

Table 1: Characteristics at enrollment in the cohort for patients with ESLD (incident cases) and without ESLD

	ESDL(n=14)	n miss	No-ESLD (n=439)	n miss	All (n=453)	n miss	p*
Demographic/general							
Male, n (%)	11 (79)	0	350 (80)	1	361 (80)	1	Ns
Age (median, IQR)	41 (40-45)	0	44 (39-49)	3	43 (39-48)	3	Ns
Education: elementary or less (n,%)	9 (64)	0	327 (76)	7	336 (75)	7	Ns
Income <1,000$/month n(%)	8 (57)	0	260 (60)	7	268 (60)	7	Ns
Deaths, n (%)	2 (14)	0	18 (4)	0	20 (4)	0	Ns
Weight	67 (61-75)	1	71 (63-81)	42	71 (63-81)	43	Ns
Hemoglobin g/L, median (IQR)	150 (118-161)	0	143 (132-152)	3	143 (132-153)	3	Ns
Calendar year of enrollment <2005; n (%)	11 (79)	0	161 (37)	0	172 (38)	0	0.003[f]
HIV infection							
AIDS diagnosis (n, %)	5 (36)	0	93 (22)	19	98 (23)	19	Ns
Duration of HIV infection, years; median (IQR)	16 (15-21)	1	19 (13-25)	31	19 (13-25)	32	Ns
CD4[2], cells/mL (median, IQR)	270 (176-373)	0	381 (254-536)	7	380 (247-531)	7	0.029[w]
Nadir CD4, cells/mL (median, IQR)	150 (104-316)	0	180 (79-300)	26	180 (79-301)	26	Ns
VL log 10 copies/mL (median, IQR)	1.7 (1.7-3.1)	2	1.7 (1.7-3.5)	10	1.7 (1.7-3.5)	12	Ns
Continuing or interrupting ART: n(%)	11 (79)	0	345(79)	1	256 (79)	1	Ns
Starting ART, n (%)	1(7)	0	35 (8)	1	36 (8)	1	

* Chi-square; f: Fisher's Exact Test, w: Wilcoxon 2 samples

[2] - when comparing CD4 <380 vs ≥ 380cells/mm^3, Fisher's test p=0.031

Table 1 continuation

	ESDL(n=14)	n miss	No-ESLD (n=439)	n miss	All (n=453)	n miss	p*
HCV infection							
HCV RNA+ (n, %)	13[3] (93)	0	321 (86)	67	334 (87)	67	Ns
Genotype 3, n (%)	4 (33)	2	67 (20)	106	71 (21)	108	Ns
Genotype 1, n (%)	7 (58)	2	238 (71)	106	245 (71)	108	Ns
Duration HCV infection-years; median (IQR)	16 (15-21)	1	19 (13-25)	39	19 (13-25)	40	Ns
HCV treatment ever, n (%)	1 (7)	0	61 (14)	6	62 (14)	6	Ns
Infection via transfusion[4],n (%)	3 (21)	0	29 (7)	29	32 (8)	29	0.080*
Infection via IDU	9 (64)	0	303 (74)	29	312 (74)	29	Ns
Co morbidities							
Diabetes (n, %)	1(7)	0	20 (5)	6	21 (5)	6	Ns
Glucose[5] (not fasting)	4.9 (3.7-5.7)	0	5.0 (4.5-5.6)	22	5.0 (4.5-5.6)	22	Ns
Hypercholesterolemia	0	2	28 (6)	14	28 (6)	16	Ns
Total Cholesterol [6]mmol/L median (IQR)	4.2 (3.7-4.9)	0	4.1 (3.6-4.9)	64	4.1 (3.6-4.9)	64	Ns
Creatinine µmol/L median (IQR)	85 (75-87)	1	74 (64-84)	17	74 (65-85)	18	0.025[w]
Estimated Clearance Cr Median (IQR)	102.9 (81.1-117.0)	1	111.2 (93.7-132.8)	58	110.8 (93.3-131.8)	59	0.047[w]

* Chi-square; f: Fisher's Exact Test, w: Wilcoxon 2 samples

[3] - 1 patient who developed ESLD had RNA negative at baseline as was treated for HCV before entering the cohort.
[4] - no other route of infection is associated with ESLD
[5] - insulin and HOMA_ir: results only for 7 patients with ESLD and only 1 of them is fasting. In general the 7 have high insulin and HOMA_ir
[6] - Also HDL, LDL and triglicerides non significant

Table 1 continuation

	ESDL(n=14)	n miss	No-ESLD (n=439)	n miss	All (n=453)	n miss	pTest*
Hepatic indices							
AST-U/L , median (IQR)	61 (48-69)	1	43 (29-68)	21	45 (29-68)	22	Ns
APRI[§]; median (IQR)	1.1 (0.61-1.24)	1	0.59 (0.36-1.1)	24	0.59 (0.38-1.17)	25	0.031
APRI >1; n(%)	8 (62)	1	120 (29)	24	128 (29)	25	0.03[f]
Platelets 10^9/L; median (IQR)	160 (140-237)	0	207 (165-253)	4	206 (163-252)	4	0.08
lnAFP µg/L, median (IQR)	1.06 (0.92-1.90)	1	1.25 (0.79-1.72)	117	1.25 (0.79-1.72)	118	Ns
Albumin g/L, median (IQR)	40 (38-44)	0	41 (38-43)	35	41 (38-43)	35	Ns
Total bilirubin µmol/L, median (IQR)	16.3 (12.8-23)	1	13 (9-19)	12	13 (9-19)	13	Ns
Fib_4 (age*AST/plts*ALT)	1.9 (1.2-3.1)	1	1.3 (1.0-1.9)	31	1.3 (1.0-1.9)	32	0.060
Risk factors (drugs)							
Hazardous or moderate past alcohol use, n (%)	4 (57)	7	158 (45)	84	162 (45)	91	Ns
Reduced alcohol use after knowing HCV; n(%)					92 (41)	228	
IDU ever, n (%)	10 (71)	0	351 (80)	2	360 (80)	1	Ns
IDU in the last 6 months, n (%)	5 (38)	1	145 (34)	13	150 (34)	14	Ns
Smoke ever, n (%)	13 (93)	0	401 (92)	5	414 (92)	5	Ns
Smoke in the last 6 months, n (%)	12 (86)	0	325 (79)	25	337 (79)	25	Ns
Marijuana smoke[7]; N (%)	10 (71)	0	230 (53)	7	240 (54)	7	Ns

* Chi-square; f: Fisher's Exact Test, w: Wilcoxon 2 samples.

[7] ~60% of the users, report to smoke marijuana to relieve symptoms/increase appetite, in both groups, §**APRI** Aspartate-aminotransferase (AST) to platelets ratio

In univariate analyses baseline factors associated with development of ESLD were APRI* > 1 (p=0.02), CD4 < 350 cells/mL (p=0.06) and haemoglobin <145g/L (p=0.05). In the multivariate analysis, controlling for sex, age, and duration of HCV infection, only APRI > 1 (aOR 3.49, 95% CI 1.10-11.06; p=0.03) and CD4 < 350 cells/mL (aOR, 3.78, 95% CI: 1.002-14.27; p=0.05) were associated with ESLD.

*APRI: Aspartate-aminotransferase (AST) to platelets ratio

Table 2. Predictors of developing ESLD: Univariate and Multivariate logistic Regression Analyses

Variables	Unadjusted OR (95%CI)	Adjusted OR (95%CI)*
Age (≥45years)	2.5 (0.7- 8.9)	2.6 (0.6 -10.1)
Sex (male)	1.0 (0.3 - 3.6)	1.4 (0.3 - 6.6)
AIDS diagnosis	2.0 (0.6- 6.0)	
Duration of HCV infection (in 10years)	1.0 (0.5 – 1.8)	0.7 (0.1 - 8.6)
Creatinine (in 10 umol/L)	1.0 (1.0 - 1.01)	
APRI > 1	**3.9 (1.2 - 12.1)**	**3.49 (1.10-11.6)**
CD4 < 350 cells/mL	**3.1 (1.0-10.1)**	**3.78 (1.002-14.27)**
Hemoglobin >145g/L	0.3 (0.1 - 1.0)	

***Adjusted for sex, age, duration of HCV infection, APRI and CD4

Conclusion and next steps

Although the study is based on a small number of outcomes over a relatively short follow-up, the preliminary results suggest that among the hepatic function markers APRI is the most sensitive for prediction of ESLD. Also immunological recovery may play an important protective role against the development ESLD.

The subsequent analysis, including the updated cohort (950 patients) and a longer follow up time, will allow us to validate these and other potential predictors of ESLD.

Epidemiology and management of gastroenteritis

Preface:
This is the third observational study. It was useful to acquire experience in how to conduct studies using retrospective cohort data, collected not through a network o hospitals or universities, but at the ambulatory level, throughout the country. It was also instructive experience on how such a cohort can be realized, which are it possible utilizations, its advantages and limitations.

Epidemiology and management of acute gastroenteritis (AGE) in the paediatric population in Italy, an observational retrospective study.

This work has resulted in a paper submitted for publication in an international journal and is under revision. Authors of the paper are:

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Introduction

Acute gastroenteritis (AGE) is one of the most frequent paediatric pathologies and each year it causes over one million deaths globally (55, 56).

In resources-rich settings gastroenteritis is one of the most frequent causes for doctor consultations as well as access to the emergency department, representing a high demand on the heath care systems (57). A study in 7 European countries (58) showed that every year 10-12% of children under the age of 5 gets AGE. Similarly, in the US 1/1.3 child had at least 1 outpatient visit for diarrhoea by age 5 in recent years (59).

Rotavirus gastroenteritis is the most frequent cause of severe AGE in children (60). Two attenuated Rotavirus vaccines (Rotarix™, GlaxoSmithKline Biologicals; RotaTeq™, Merck & Co),

have been shown to be very effective in preventing rotavirus diarrhoea (61, 62) and their use on large scale is expected to significantly reduce the incidence of rotavirus AGE. However, this would have no impact on other causes of AGE, leaving the need for treatment that, independently of the causative infective agent, can prevent the risk of dehydration and hospitalisation in severe forms and younger age group. Current treatment of AGE is usually symptomatic and consists of the correct administration of fluids to decrease the risk of dehydration; antibiotics are generally contraindicated (38).

To assess the impact of treatment on management of AGE in children, data on burden of disease, current management and outcome of gastroenteritis in the paediatric population is needed. While hospital and emergency department based studies can report on most severe case, national wide data at ambulatory level are needed to estimate the outpatient incidence of AGE and burden of disease on health system. In Italy, primary health care system devoted to children up to the age of 14 years includes approximately 6000 family paediatricians (FP) throughout the country. PEDIANET (www.pedianet.it) is a network established to provide data for epidemiological and clinical research collected by FP. Data collected through the PEDIANET has been previously employed in several epidemiological studies (63-66). A total of 300 FP have already participated to collect data on incidence and clinical management of diseases, utilized in epidemiological studies. The referral cohort of children registered under the care of participating paediatricians is approximately of 130,000. Participating FP work throughout the whole country and are present in each macro-region (Central, Insular, North and South). In particular they are spread in 15 out of 20 regions, 7 in Northern Italy, 3 in Central, 3 in the South and the 2 islands.

Objectives of this study were to estimate the incidence of AGE in Italian children, its clinical presentation, management and outcome in outpatients setting.

Methods

This study is a retrospective cohort study on data collected in Italy through PEDIANET network. For those patients whose parents/guardians have given their inform consent, data were automatically collected by use of specific software linked to electronic patient records compiled by FP. Data, collected and handled anonymously in compliance with Italian law, were: disease diagnoses, symptoms, prescriptions, certificates, results of medical examinations, referrals and hospitalisations. They are periodically sent by internet to a central server in Padua where they are validated and analyzed together with the Departments of Epidemiology and Biostatistics and Medical Informatics, Erasmus University Medical Center, Rotterdam, The Netherlands.

Population and case definition:

All children aged 0-12 years registered with a FP who sent information to the network during the period from 1 January 2002 to 31 March 2008 were included in this analysis. AGE was defined as acute diarrhoea -at least 3 watery stools in 24 hours- without chronic abdominal pathology as malformations, celiac disease, Hirschsprung, or malabsorption. The children were enrolled either at date of registration with the paediatrician, or start of study period (whichever came first) until death, moving out, reaching 12 years of age or the end of the study (whichever occurred last).

Cases were identified retrospectively using electronic queries (ICD 8.6 and text string searches in free text boxes). All potential episodes of AGE thus found were individually validated.

Visits for AGE were considered to belong to one episode if they occurred within one month of the initial visit; thus one patient could have more than one episode of AGE if they had occurred more than 30 days apart.

Variables analyzed for each episode of AGE were age, sex, area of residence, clinical presentation, prescriptions given, number of consultations per episode, accesses to emergency department, hospitalizations. Fever was considered as temperature >38℃.

Statistical analysis:

The incidence of AGE was expressed as number of events per 100 person-years (PY) of follow-up and calculated by age, sex and calendar year. Frequencies of AGE, clinical presentation, treatment and outcome were presented with 95% confidence intervals. Univariate and multivariate analysis were used to test association between patients' variables and AGE, its presentation and outcomes. Differences were considered significant if associated $p<0.05$.

Results

Incidence of AGE:

A total of 121,429 children aged between 0 and 12 years, for whom data were sent during the study period (January 2002 to March 2008), were included in the study. Children were followed by 84 FP in 10 regions: Abruzzo (3), Campania (12), Emilia Romagna (9), Friuli Venezia Giulia (6), Lombardia (12), Marche (10), Piemonte (3), Sardegna (5), Sicilia (7), Veneto (17).

During the study period there were 33,745 cases of AGE in a total of 463,909 person years (table 1). The average (SD) age of cases was 4.3 (3.0) years. The absolute incidence of AGE in the years 2002-07 was 7.2 per 100 person years (95% CI 7.1-7.4) with a range between 6.0 and 8.2 per 100PY in different time periods (Table 1).

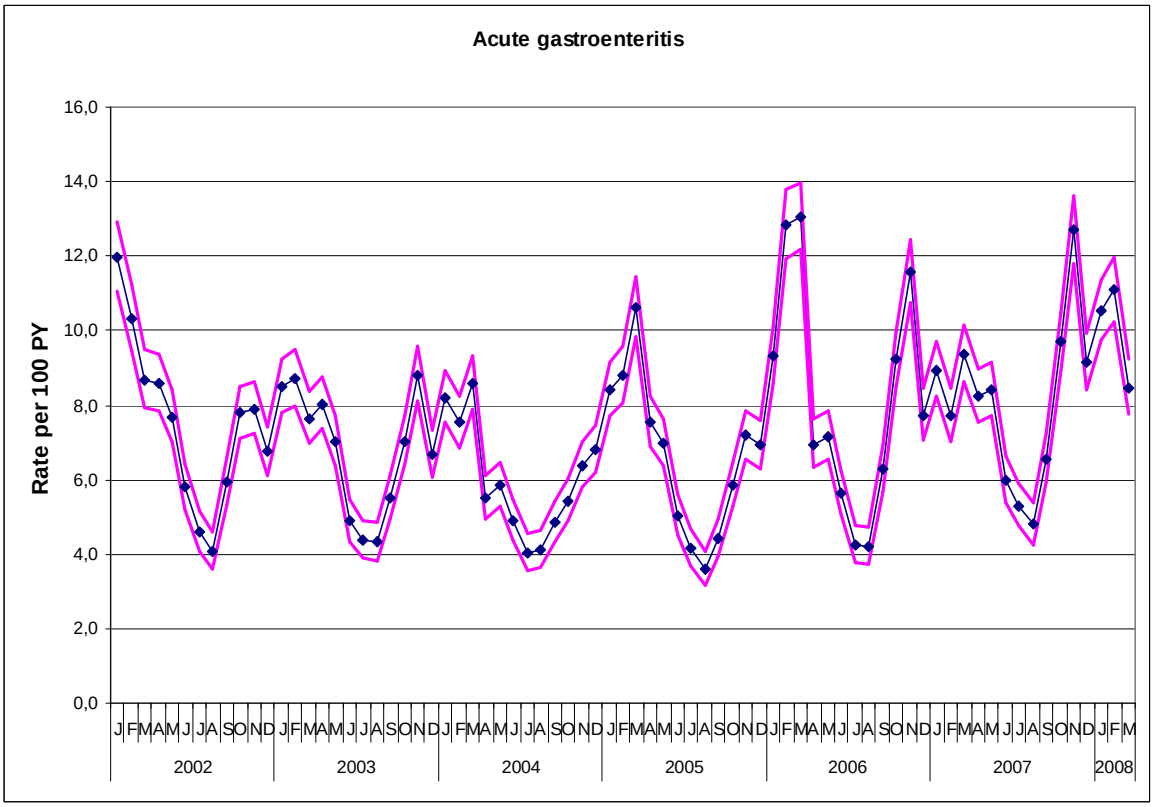
Table 1: Person-years, cases and rates of acute gastroenteritis (AGE) by calendar year

	2002	**2003**	**2004**	**2005**	**2006**	**2007**	***2008****	**Total**
Person-years, n (%)	70508 (15.2)	74817 (16.1)	76197 (16.4)	76986 (16.6)	74911 (16.1)	72696 (15.7)	*17795* *(3.8)*	463909 (100)
AGE cases, n (%)	5230 (15.5)	5071 (15.0)	4583 (13.6)	5097 (15.1)	6112 (18.1)	5871 (17.4)	*1781* *(5.3)*	33745 (100)
AGE rate, per 100PY (95%CI)	7.4 (7.2-7.6)	6.8 (6.6-7.0)	6.0 (5.8- 6.2)	6.6 (6.4-6.8)	8.2 (8.0-8.4)	8.1 (7.9-8.3)	*10.0* *(9.6-10.5)*	7.3 (7.2-7.4)

* 1st trimester only

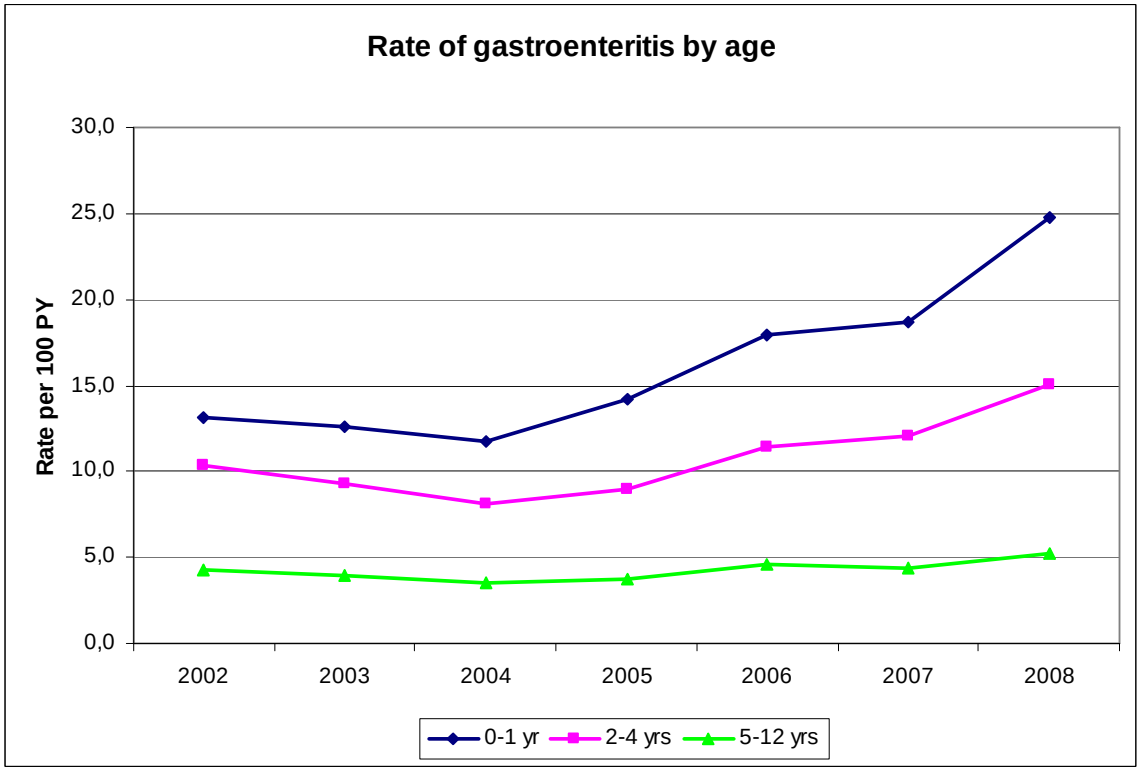
The incidence of AGE in the first 3 months of 2008 was 10 per 100PY (95%CI 9.6 – 10.5) which is comparable to the incidence in the same periods of the preceding two years (Table 1). AGE peaked in winter months (figure 1) and had significantly higher incidence for males (7.6/100 person years; 95% CI 7.4-7.7) than for females (7.0/100 PY; 95% CI 6.9-7.1), with a RR for females of 0.92 (95% CI 0.90-0.94).

Figure 1: Rate of acute gastroenteritis (95%CI) by month.



In all years, the incidence of AGE was higher in younger age groups (figure 2); cumulative incidence (2002-2007) was 14.5 per 100 PY (95% CI 14.0-15.4) in children <1 year; 9.9 per 100 PY (95% CI 9.5-10.5) in children aged 2-4 years and 4.1 per 100 PY (95% CI 3.9-4.2) in children >4 years old (figure 2).

Figure 2: Rate of gastroenteritis by age group,
Note: for 2008 only months January-March



Clinical presentation

A total of 30994 (92%) children with AGE had diarrhoea; other most frequent symptoms were vomiting and fever. Associated respiratory tract symptoms were reported in 4466 (13%) children (table 2) and were more common in younger children while abdominal pain was reported more frequently in older children (both $p<0.05$) (table 2).

Table 2: Cases and presentation symptoms of gastroenteritis by age group

	Age (in years)			
	0-1	**2-4**	**5-12**	**Total**
N° of cases	10109	12258	11378	33745
(%)	(30)	(36)	(34)	(100)
Symptoms*, n (%)				
Diarrhoea	9554(94.5%)	11221 (91.5%)	10219 (89.8%)	30994 (91.8)
Fever	2317 (22.9%)	2767 (22.6%)	2127 (18.7%)	7211 (21.4)
Vomiting	2700 (26.7%)	4350 (35.5%)	3648 (32.1%)	10698 (31.7)
Abdominal pain	272 (2.7%)	1325 (10.8%)	2748 (24.2%)	4345 (12.9)
Respiratory symptoms	1682 (16.6%)	1798 (14.7%)	986 (8.7%)	4466 (13.2)
Urinary Tract Infection	18 (0.2%)	9 (0.1%)	4 (0.0%)	31 (0.1)

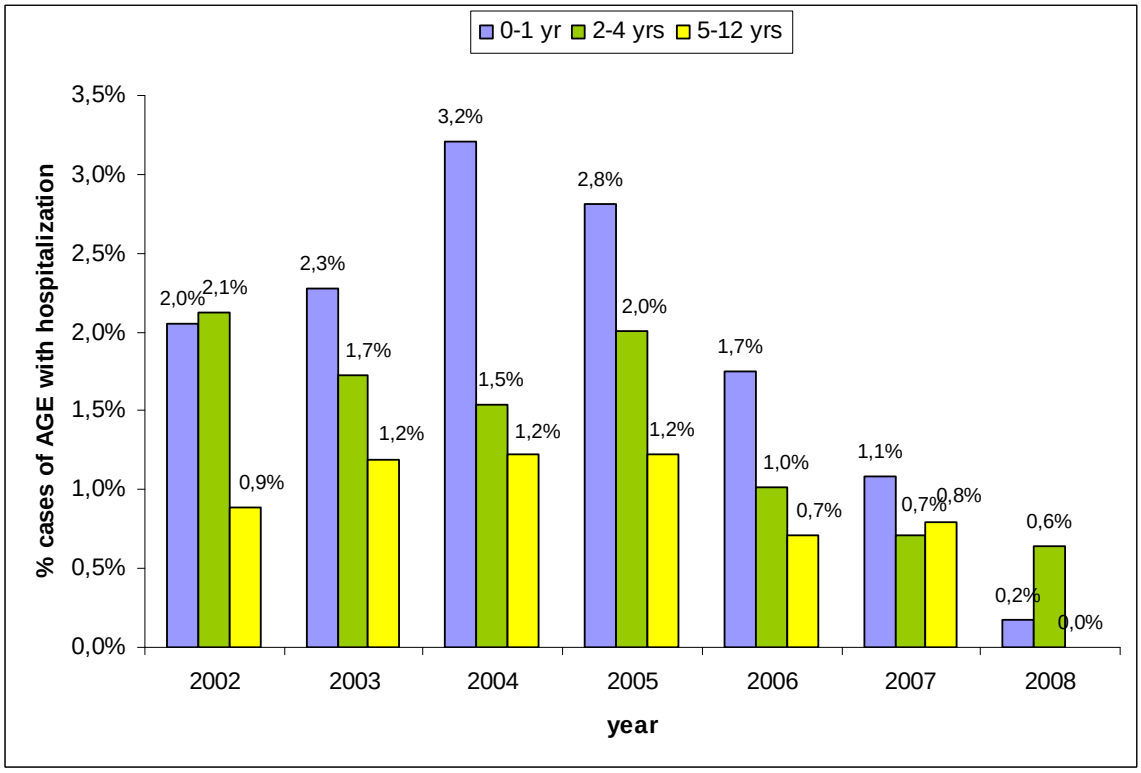
***** Symptoms within 7 days from the diagnosis

Burden on health services and management

The burden of the disease on the family paediatrician (FP) workload was evaluated indirectly using the number of appointments per episode of AGE which were connected to the AGE episode itself. Twenty percent of children with AGE returned to see their family paediatrician within 7 days of diagnosis and 40% within 15 days of the diagnosis (with an average of 1.4 consultations per episode of AGE). Stool sample was taken for 5.5% of children with AGE, resulting in 1,867 specimens. For most (96%) of these samples there was no recorded result; among the reported results, 75% were negative. The taking of a stool sample was associated with diarrhoea in all age groups, but multivariate analysis showed no association with age, sex or other symptoms.

The FP reported that a total of 634 children (1.9% of all cases of AGE) required further care: 145 children (0.4%) had a visit in the emergency departments (ED) and 489 children (1.5%) required hospitalisation (figure 3).

Figure 3: Hospitalization by age and year
Note: for 2008 only months January-March



Both access to the ED and hospitalizations were the highest for children younger than 1 year of age ($p<0.05$). The main reasons for accessing the emergency department within 7 days of diagnosis of AGE were persistent diarrhoea or dehydration. The vast majority of hospitalizations were for symptoms or consequences of the illness itself.

Between January 2002 and March 2008, 11,562 (34.3%) out of the 33,745 cases were given at least one prescription: 90% at diagnosis and 10% within 1 week, at the second visit. Children less than 1 year of age were less likely to receive drugs than children in the older age groups ($p<0.05$). The most commonly prescribed drugs were prokinetics (30.7% of all cases who received treatment) and anti-diarrhoeal probiotics (21.3%). Domperidone was the single most commonly prescribed drug (23.1% of all cases who had treatment). Other anti-diarrhoeal drugs, commonly used in adults, were rarely used: Loperamide, was used in 25 cases, 0.2% of the total treated. Racecadotril (Tiorfix), an antisecretory drug, was used in 5% of all cases of AGE in infants <1 year

old. In 13.5% of treated case, antibiotics were prescribed (amoxicillin; amoxicillin-clavulanic acid; trimethoprim-cotrimoxazole) (Table 3).

Table 3: Drugs most frequently prescribed: number of prescriptions and percentage on total cases with at least one drug prescribed for each age group (if not otherwise specified). One case could receive more than one prescription.

***** Amoxicillin, Amoxicillin/Clavulanate, Trimetoprim/Sulfametoxazole.

** Bacillus Clausii, Bifidobacterium Bifidum / Lactobacillus Acidiphilus, Saccaromices Boulardii, others.

Drugs	Age in years 0-1		2-4		5-12		Total	
	n	%	n	%	n	%	n	%
Prokinetics								
Domperidone	521	17,0	1069	24,4	1082	26.2	2672	23.1
Metoclopramide	172	5,6	397	9,1	315	7,6	884	7.6
Probiotics*	785	25.7	903	20.6	774	18.8	2462	21.3
Antibiotics**	484	15.8	621	14.2	457	11.1	1562	13.5
Analgesics								
Paracetamol	150	4,9	214	4,9	217	5,3	581	5.0
Electrolytes and vitamins								
Sodium Citrate/Potassium Citrate/Vitamin C Complex	81	2,6	217	5,0	247	6,0	545	4.7
Others								
Other drugs within the Anatomical and Chemical Classification	1490	48,7	1991	45,5	1946	47,2	5427	46.9
Others	770	25,2	1167	26,6	917	22,2	2854	24.7
Cases receiving at least one prescription: n,% on total AGE cases	3057	30.2	4379	35.7	4126	36.3	11562	34.3

Discussion and conclusion

The network utilized in this study, PEDIANET, offered a unique setting to realize a large observational study and to acquire information on the epidemiology of AGE, its presentation and its management in pre-hospital setting throughout the country,

Incidence of AGE seen by FP was 7.2/100PY (range 6.0 to 8.2/100PY) during a period of 5 years (2002-2007) with an average of 1.4 consultation/episode. Consistently with previous studies on gastroenteritis and rotavirus infections (38, 67-70) incidence of AGE was higher in winter, in younger children and in male patients.

These results are comparable with an European study where in 7 countries overall annual incidence of AGE seen at the primary care/emergency/hospital level was between 4 and 17 cases/100 children (58). Similarly, in a large study in the US, annual rate of ambulatory visits in privately insured children <5 years was 13.3/100 (59). Other studies, based on AGE cases reported by the FP or general practitioner have found a higher incidence: 21% in Italy (71); 15.5% in UK (72) and 25.2% in Germany (73). This could be in part explained by the older age of the children included in our study (up to 12 years) as compared with others. When looking only at young children (0-4 years old) in our study, the incidence was 11.6 per 100 PY (95%CI 11.5 – 11.7). In addition, in the Italian study (71), telephonic consultations were also considered to record cases of AGE, therefore capturing also the cases that did not require a visit. Being based on a network of sentinel FP, our study gives the incidence of AGE cases seeking primary care. By design, the emergent cases, likely referring directly to ED, the mildest, not consulting any medical structure, and the one not accessing the FP, for social or other reasons not here investigated, are not considered. This leads to a possible underestimation of the overall incidence of AGE in the country, while allowing for estimation of clinically relevant AGE forms and of the burden that moderate/mild diarrhoeas have on FP workload.

In The Nederlands, deWit et al, have estimated that only 5% of patients with a AGE consults a GP (74); even assuming that in the paediatric population the percentage of AGE cases consulting a FP is higher, the incidence of AGE found in our study is expected to be lower than in community based studies, as the one realized by Guandalini et al. (75), where the higher incidence observed can be due to the fact that all cases of acute diarrhea were considered and not only those seeking medical care.

In future studies, ED records could be considered together with sentinel FP, in order to have a more complete measure of the overall incidence of diarrhoeas for which medical attention is sought.

Within the period studied, the incidence of AGE decreased temporarily in 2004 (figure 2), independently of sex and age. As most observed cases occurred during the first two years of life, the same age group with most rotavirus infection, it may be due to the decreased incidence of rotavirus observed in winter 2004 in Italy compared to other years (58). This is also supported by the fact that the incidence of AGE is always higher in the winter months when Rotavirus plays a key role in the aetiology of gastroenteritis cases.

The clinical presentation was characterised by diarrhoea associated to abdominal pain and vomit in older children (> 5 years old) and to respiratory symptoms among < 1 year olds. Association between severity of clinical presentation and management (therapy, diagnostic exams and hospital referral) was not possible as specific information on severity of disease was not available for this analysis.

A stool exam was requested for 5.5% of the cases of AGE, with no significant association with clinical or epidemiological characteristics of the patient. As the reasons for stool specimen request is not specifically collected was not possible to infer the appropriateness of the request in light of the European guidelines.

Admission to ED was reported in a small percentage (0.4%) of total cases of AGE. Nevertheless, the low number of accesses to ED could be due to underreporting to the FP of visits not resulting in hospitalization or of which the FP was not the referring doctor. The main reasons for ED access and/or hospitalization were persistent diarrhoea and dehydration. Although a relationship between severity of presentation and age has not been found in previous studies (76) the reason for a higher admission rate among younger children could be explained by a higher incidence of Rotavirus infection in this age. In this analysis however, it was not possible to ascertain the aetiology of AGE, nor a specific severity of presentation. In light of the recommendation in the European guidelines (38) and of a number of unnecessary hospitalizations previously reported in industrialized countries (77-79), further studies could be undertaken on the appropriateness of admissions. However in this study the risk of hospitalisation is relatively low as compared with other settings.

Overall there was a large use of treatments with 34.3% of patients receiving at least 1 drug (Table 3). This result is probably an underestimation as only drugs that require medical prescription were captured, leaving incomplete the information of oral re-hydrating solution and probiotics, sold over the counter. Also the reason for drug prescription was not specifically captured in the database. For these reasons is not possible to evaluate the complete treatment prescribed and whether the use of antibiotics, not recommended for uncomplicated diarrhoea, was actually due, in our sample, to concomitant respiratory infections. Even with these limitations, the overall paediatricians' prescriptions give important information on management of diarrhoeas in the outpatients' setting. Information on drugs prescription, in particular, of antibiotics and probiotics, can be useful for planning future research on management of AGE at primary health care level. Of note, in our study, the most used probiotic was *B. clausii*, not recommended in the recent guidelines (38), as evidence of efficacy is lacking (80).

Metoclopramide is a commonly used anti-nausea and anti-emetic drug, and it was commonly prescribed before 2004. A pharmacovigilance study conducted by the Ministry of Health in Italy showed that the use of metoclopramide increased the risk of extrapyramidal effects in children.

Since 2004 it is therefore contraindicated in children less than 16 years of age (81). In our analysis its use went from 20% of cases ≥1 year before 2004 to less than 5% since 2005.
We also saw a rapid increase in the use of racecadotril, an enkephalinase inhibitor that decreases the amount of fluid at an intestinal level (82). Approved in Italy for use in children in September 2007, it accounted for 52% of same class prescriptions in the first trimester of 2008.

The findings of this study are likely generalizable to the whole country as participating FPs work throughout Italy, and were present in each macro-region, even if more in the Northern. Nevertheless, in planning future studies on management and treatment of AGE at the primary care level, more balance between regions should be sought in order to account for difference in socio-economical conditions.

In conclusion, in this large study on outpatient presentation and management of AGE, we saw higher incidence in younger children and males and winter seasonality. The preliminary data on management at the primary health level (FP), as the high rate of treatments prescribed, provides bases for further studies, specifically designed to know medications usage and referral patterns used by FPs.

References for all studies

1. World Health Organization. Towards universal access: scaling up priority HIV/AIDS interventions in the health sector. Progress report, September 2009. http://wwwwhoint/hiv/pub/2009progressreport/en/indexhtml. 2009.

2. Braitstein P, Brinkhof MW, Dabis F, Schechter M, Boulle A, Miotti P, et al. Mortality of HIV-1-infected patients in the first year of antiretroviral therapy: comparison between low-income and high-income countries. Lancet. 2006 Mar 11;367(9513):817-24.

3. Egger M, May M, Chene G, Phillips AN, Ledergerber B, Dabis F, et al. Prognosis of HIV-1-infected patients starting highly active antiretroviral therapy: a collaborative analysis of prospective studies. Lancet. 2002 Jul 13;360(9327):119-29.

4. Lawn SD, Harries AD, Anglaret X, Myer L, Wood R. Early mortality among adults accessing antiretroviral treatment programmes in sub-Saharan Africa. AIDS. 2008 Oct 1;22(15):1897-908.

5. Lawn SD, Harries AD, Wood R. Strategies to reduce early morbidity and mortality in adults receiving antiretroviral therapy in resource-limited settings. Curr Opin HIV AIDS. 2010 Jan;5(1):18-26.

6. Chasombat S, Lertpiriyasuwat C, Thanprasertsuk S, Suebsaeng L, Lo YR. The National Access to Antiretroviral Program for PHA (NAPHA) in Thailand. Southeast Asian J Trop Med Public Health. 2006 Jul;37(4):704-15.

7. National AIDS Prevention and Alleviation Committee. UNGASS Country Progress Report Thailand. http://dataunaidsorg/pub/Report/2010/thailand_2010_country_progress_report_enpdf. 2009.

8. Lallemant M, Jourdain G, Le Coeur S, Kim S, Koetsawang S, Comeau AM, et al. A trial of shortened zidovudine regimens to prevent mother-to-child transmission of human immunodeficiency virus type 1. Perinatal HIV Prevention Trial (Thailand) Investigators. N Engl J Med. 2000 Oct 5;343(14):982-91.

9. Lallemant M, Jourdain G, Le Coeur S, Mary JY, Ngo-Giang-Huong N, Koetsawang S, et al. Single-dose perinatal nevirapine plus standard zidovudine to prevent mother-to-child transmission of HIV-1 in Thailand. N Engl J Med. 2004 Jul 15;351(3):217-28.

10. World Health Organization. Scaling up antiretroviral therapy in resource-limited settings : guidelines for a public health approach. http://wwwwhoint/hiv/pub/prev_care/en/ScalingUp_Epdf. 2002.

11. Hess KR. Assessing time-by-covariate interactions in proportional hazards regression models using cubic spline functions. Stat Med. 1994 May 30;13(10):1045-62.

12. Grabar S, Le Moing V, Goujard C, Egger M, Leport C, Kazatchkine MD, et al. Response to highly active antiretroviral therapy at 6 months and long-term disease progression in HIV-1 infection. J Acquir Immune Defic Syndr. 2005 Jul 1;39(3):284-92.

13. National Institute of Allergy and Infectious Diseases-Division of AIDS. Table for grading the severity of adult and pediatric adverse events. Verison 1.0, December 2004; Clarification August 2009. http://rsctech-rescom/Document/safetyandpharmacovigilance/Table_for_Grading_Severity_of_Adult_Pediatric_Adverse_Eventspdf. 2004.

14. World Health Organization. Global Databse on Body Mass Index-BMI Classification. http://appswhoint/bmi/indexjsp?introPage=intro_3html.

15. Royston P. Multiple imputation of missing values: Update of ice. The Stata Journal. 2005;5(4):527-36.

16. van Buuren S, Boshuizen HC, Knook DL. Multiple imputation of missing blood pressure covariates in survival analysis. Stat Med. 1999 Mar 30;18(6):681-94.

17. Chene G, Sterne JA, May M, Costagliola D, Ledergerber B, Phillips AN, et al. Prognostic importance of initial response in HIV-1 infected patients starting potent antiretroviral therapy: analysis of prospective studies. Lancet. 2003 Aug 30;362(9385):679-86.

18. Lohse N, Hansen AB, Pedersen G, Kronborg G, Gerstoft J, Sorensen HT, et al. Survival of persons with and without HIV infection in Denmark, 1995-2005. Ann Intern Med. 2007 Jan 16;146(2):87-95.

19. Mocroft A, Ledergerber B, Katlama C, Kirk O, Reiss P, d'Arminio Monforte A, et al. Decline in the AIDS and death rates in the EuroSIDA study: an observational study. Lancet. 2003 Jul 5;362(9377):22-9.

20. Tuboi SH, Schechter M, McGowan CC, Cesar C, Krolewiecki A, Cahn P, et al. Mortality during the first year of potent antiretroviral therapy in HIV-1-infected patients in 7 sites throughout Latin America and the Caribbean. J Acquir Immune Defic Syndr. 2009 Aug 15;51(5):615-23.

21. Chasombat S, McConnell MS, Siangphoe U, Yuktanont P, Jirawattanapisal T, Fox K, et al. National expansion of antiretroviral treatment in Thailand, 2000-2007: program scale-up and patient outcomes. J Acquir Immune Defic Syndr. 2009 Apr 15;50(5):506-12.

22. Ferradini L, Laureillard D, Prak N, Ngeth C, Fernandez M, Pinoges L, et al. Positive outcomes of HAART at 24 months in HIV-infected patients in Cambodia. AIDS. 2007 Nov 12;21(17):2293-301.

23. Etard JF, Ndiaye I, Thierry-Mieg M, Gueye NF, Gueye PM, Laniece I, et al. Mortality and causes of death in adults receiving highly active antiretroviral therapy in Senegal: a 7-year cohort study. AIDS. 2006 May 12;20(8):1181-9.

24. Lawn SD, Myer L, Harling G, Orrell C, Bekker LG, Wood R. Determinants of mortality and nondeath losses from an antiretroviral treatment service in South Africa: implications for program evaluation. Clin Infect Dis. 2006 Sep 15;43(6):770-6.

25. Mugyenyi P, Walker AS, Hakim J, Munderi P, Gibb DM, Kityo C, et al. Routine versus clinically driven laboratory monitoring of HIV antiretroviral therapy in Africa (DART): a randomised non-inferiority trial. Lancet. Jan 9;375(9709):123-31.

26. Laurent C, Ngom Gueye NF, Ndour CT, Gueye PM, Diouf M, Diakhate N, et al. Long-term benefits of highly active antiretroviral therapy in Senegalese HIV-1-infected adults. J Acquir Immune Defic Syndr. 2005 Jan 1;38(1):14-7.

27. De Beaudrap P, Etard JF, Ecochard R, Diouf A, Dieng AB, Cilote V, et al. Change over time of mortality predictors after HAART initiation in a Senegalese cohort. Eur J Epidemiol. 2008;23(3):227-34.

28. Thai S, Koole O, Un P, Ros S, De Munter P, Van Damme W, et al. Five-year experience with scaling-up access to antiretroviral treatment in an HIV care programme in Cambodia. Trop Med Int Health. 2009 Sep;14(9):1048-58.

29. May M, Boulle A, Phiri S, Messou E, Myer L, Wood R, et al. Prognosis of patients with HIV-1 infection starting antiretroviral therapy in sub-Saharan Africa: a collaborative analysis of scale-up programmes. Lancet. Aug 7;376(9739):449-57.

30. WHO and UNAIDS. Guidance on provider-initiated HIV testing and counselling in health facilities 2007 [updated 2007; cited 02 November 2010]; Available from: http://whqlibdoc.who.int/publications/2007/9789241595568_eng.pdf.

31. Importance of baseline prognostic factors with increasing time since initiation of highly active antiretroviral therapy: collaborative analysis of cohorts of HIV-1-infected patients. J Acquir Immune Defic Syndr. 2007 Dec 15;46(5):607-15.

32. Lundgren JD, Mocroft A. Anemia and survival in human immunodeficiency virus. Clin Infect Dis. 2003;37 Suppl 4:S297-303.

33. Volberding PA, Levine AM, Dieterich D, Mildvan D, Mitsuyasu R, Saag M. Anemia in HIV infection: clinical impact and evidence-based management strategies. Clin Infect Dis. 2004 May 15;38(10):1454-63.

34. Mocroft A, Kirk O, Barton SE, Dietrich M, Proenca R, Colebunders R, et al. Anaemia is an independent predictive marker for clinical prognosis in HIV-infected patients from across Europe. EuroSIDA study group. AIDS. 1999 May 28;13(8):943-50.

35. Lundgren JD, Mocroft A, Gatell JM, Ledergerber B, D'Arminio Monforte A, Hermans P, et al. A clinically prognostic scoring system for patients receiving highly active antiretroviral therapy: results from the EuroSIDA study. J Infect Dis. 2002 Jan 15;185(2):178-87.

36. Bisson GP, Nthobatsong R, Thakur R, Lesetedi G, Vinekar K, Tebas P, et al. The Use of HAART Is Associated With Decreased Risk of Death During Initial Treatment of Cryptococcal Meningitis in Adults in Botswana. J Acquir Immune Defic Syndr. 2008 Sep 2.

37. Cornell M, Myer L, Kaplan R, Bekker LG, Wood R. The impact of gender and income on survival and retention in a South African antiretroviral therapy programme. Trop Med Int Health. 2009 Jul;14(7):722-31.

38. Bisson GP, Gaolathe T, Gross R, Rollins C, Bellamy S, Mogorosi M, et al. Overestimates of survival after HAART: implications for global scale-up efforts. PLoS ONE. 2008;3(3):e1725.

39. Srasuebkul P, Lim PL, Lee MP, Kumarasamy N, Zhou J, Sirisanthana T, et al. Short-term clinical disease progression in HIV-infected patients receiving combination antiretroviral therapy: results from the TREAT Asia HIV observational database. Clin Infect Dis. 2009 Apr 1;48(7):940-50.

40. Jarrin I, Geskus R, Bhaskaran K, Prins M, Perez-Hoyos S, Muga R, et al. Gender differences in HIV progression to AIDS and death in industrialized countries: slower disease progression following HIV seroconversion in women. Am J Epidemiol. 2008 Sep 1;168(5):532-40.

41. Lemly DC, Shepherd BE, Hulgan T, Rebeiro P, Stinnette S, Blackwell RB, et al. Race and sex differences in antiretroviral therapy use and mortality among HIV-infected persons in care. J Infect Dis. 2009 Apr 1;199(7):991-8.

42. Nicastri E, Leone S, Angeletti C, Palmisano L, Sarmati L, Chiesi A, et al. Sex issues in HIV-1-infected persons during highly active antiretroviral therapy: a systematic review. J Antimicrob Chemother. 2007 Oct;60(4):724-32.

43. Im-Em W. Mortality trends and levels to verify the AIDS epidemic in Thailand: Analysis from death registration statistics, 1984-1997. Nakhon Pathom: Mahidol University, Institute for Population and Social Research; 1999.

44. Boyd MA. Improvements in antiretroviral therapy outcomes over calendar time. Curr Opin HIV AIDS. 2009 May;4(3):194-9.

45. Brinkhof MW, Dabis F, Myer L, Bangsberg DR, Boulle A, Nash D, et al. Early loss of HIV-infected patients on potent antiretroviral therapy programmes in lower-income countries. Bull World Health Organ. 2008 Jul;86(7):559-67.

46. Boulle A, Van Cutsem G, Hilderbrand K, Cragg C, Abrahams M, Mathee S, et al. Seven-year experience of a primary care antiretroviral treatment programme in Khayelitsha, South Africa. AIDS. Feb 20;24(4):563-72.

47. Rosen S, Fox MP, Gill CJ. Patient retention in antiretroviral therapy programs in sub-Saharan Africa: a systematic review. PLoS Med. 2007 Oct 16;4(10):e298.

48. Delpierre C, Cuzin L, Lauwers-Cances V, Datta GD, Berkman L, Lang T. Unemployment as a risk factor for AIDS and death for HIV-infected patients in the era of highly active antiretroviral therapy. Sex Transm Infect. 2008 Jun;84(3):183-6.

49. DeSilva MB, Merry SP, Fischer PR, Rohrer JE, Isichei CO, Cha SS. Youth, unemployment, and male gender predict mortality in AIDS patients started on HAART in Nigeria. AIDS Care. 2009 Jan;21(1):70-7.

50. Lanoy E, May M, Mocroft A, Phillip A, Justice A, Chene G, et al. Prognosis of patients treated with cART from 36 months after initiation, according to current and previous CD4 cell count and plasma HIV-1 RNA measurements. AIDS. 2009 Oct 23;23(16):2199-208.

51. Stringer JS, McConnell MS, Kiarie J, Bolu O, Anekthananon T, Jariyasethpong T, et al. Effectiveness of non-nucleoside reverse-transcriptase inhibitor-based antiretroviral therapy in women previously exposed to a single intrapartum dose of nevirapine: a multi-country, prospective cohort study. PLoS Med.7(2):e1000233.

52. Chintu N, Giganti MJ, Putta NB, Sinkala M, Sadoki E, Stringer EM, et al. Peripartum nevirapine exposure and subsequent clinical outcomes among HIV-infected women receiving antiretroviral therapy for at least 12 months. Trop Med Int Health. Jul;15(7):842-7.

53. Jourdain G, Ngo-Giang-Huong N, Le Coeur S, Bowonwatanuwong C, Kantipong P, Leechanachai P, et al. Intrapartum exposure to nevirapine and subsequent maternal responses to nevirapine-based antiretroviral therapy. N Engl J Med. 2004 Jul 15;351(3):229-40.

54. Palella FJ, Jr., Delaney KM, Moorman AC, Loveless MO, Fuhrer J, Satten GA, et al. Declining morbidity and mortality among patients with advanced human immunodeficiency virus infection. HIV Outpatient Study Investigators. N Engl J Med. 1998 Mar 26;338(13):853-60.

55. Black RE, Morris SS, Bryce J. Where and why are 10 million children dying every year? Lancet. 2003 Jun 28;361(9376):2226-34.

56. Kosek M, Bern C, Guerrant RL. The global burden of diarrhoeal disease, as estimated from studies published between 1992 and 2000. Bull World Health Organ. 2003;81(3):197-204.

57. Forster J, Guarino A, Parez N, Moraga F, Roman E, Mory O, et al. Hospital-based surveillance to estimate the burden of rotavirus gastroenteritis among European children younger than 5 years of age. Pediatrics. 2009 Mar;123(3):e393-400.

58. Van Damme P, Giaquinto C, Huet F, Gothefors L, Maxwell M, Van der Wielen M. Multicenter prospective study of the burden of rotavirus acute gastroenteritis in Europe, 2004-2005: the REVEAL study. J Infect Dis. 2007 May 1;195 Suppl 1:S4-S16.

59. Cortes JE, Curns AT, Tate JE, Parashar UD. Trends in healthcare utilization for diarrhea and rotavirus disease in privately insured US children <5 years of age, 2001-2006. Pediatr Infect Dis J. 2009 Oct;28(10):874-8.

60. Parashar UD, Gibson CJ, Bresse JS, Glass RI. Rotavirus and severe childhood diarrhea. Emerg Infect Dis. 2006 Feb;12(2):304-6.

61. Ruiz-Palacios GM, Perez-Schael I, Velazquez FR, Abate H, Breuer T, Clemens SC, et al. Safety and efficacy of an attenuated vaccine against severe rotavirus gastroenteritis. N Engl J Med. 2006 Jan 5;354(1):11-22.

62. Vesikari T, Matson DO, Dennehy P, Van Damme P, Santosham M, Rodriguez Z, et al. Safety and efficacy of a pentavalent human-bovine (WC3) reassortant rotavirus vaccine. N Engl J Med. 2006 Jan 5;354(1):23-33.

63. Menniti-Ippolito G, Raschetti R, Da Cas R, Giaquinto C, Cantarutti L. Active monitoring of adverse drug reactions in children. Italian Paediatric Pharmacosurveillance Multicenter Group. Lancet. 2000 May 6;355(9215):1613-4.

64. Nicolosi A, Sturkenboom M, Mannino S, Arpinelli F, Cantarutti L, Giaquinto C. The incidence of varicella: correction of a common error. Epidemiology. 2003 Jan;14(1):99-102.

65. Sturkenboom M, Nicolosi A, Cantarutti L, Mannino S, Picelli G, Scamarcia A, et al. Incidence of mucocutaneous reactions in children treated with niflumic acid, other nonsteroidal antiinflammatory drugs, or nonopioid analgesics. Pediatrics. 2005 Jul;116(1):e26-33.

66. Sturkenboom M S-GM, Picelli G, Scamarcia A, Fregonese F, Cantarutti L, Franco E and Giaquinto C. . Incidence and Outcomes of Acute Gastroenteritis in Italian Children The Pediatric Infectious Disease Journal, . 2008 Jan 2008;Volume 27 (1 Supplement):pp S42-S7.

67. Grassi T, De Donno A, Guido M, Gabutti G. The epidemiology and disease burden of rotavirus infection in the Salento peninsula, Italy. Turk J Pediatr. 2008 Mar-Apr;50(2):132-6.

68. Grimwood K, Huang QS, Cohet C, Gosling IA, Hook SM, Teele DW, et al. Rotavirus hospitalisation in New Zealand children under 3 years of age. J Paediatr Child Health. 2006 Apr;42(4):196-203.

69. Roman E, Wilhelmi I, Colomina J, Villar J, Cilleruelo ML, Nebreda V, et al. Acute viral gastroenteritis: proportion and clinical relevance of multiple infections in Spanish children. J Med Microbiol. 2003 May;52(Pt 5):435-40.

70. Zuccotti G, Meneghin F, Dilillo D, Romano L, Bottone R, Mantegazza C, et al. Epidemiological and clinical features of rotavirus among children younger than 5 years of age hospitalized with acute gastroenteritis in Northern Italy. BMC Infect Dis.10:218.

71. Ansaldi F, Lai P, Valle L, Riente R, Durando P, Sticchi L, et al. Burden of rotavirus-associated and non-rotavirus-associated diarrhea among nonhospitalized individuals in central Italy: a 1-year sentinel-based epidemiological and virological surveillance. Clin Infect Dis. 2008 Mar 15;46(6):e51-5.

72. Djuretic T RM, Gay N, et al. An estimate of the proportion of diarrhoeal disease episodes seen by general practitioners attributable to rotavirus in children under 5 y of age in England and Wales. Acta PEdiatr Suppl. 1999;426:38-41.

73. Ehlken B LB, Karmaus W. Prospective population-based study on rotavirus disease in Germany. Acta Paediatr. 2002;91:769:75.

74. de Wit MA, Kortbeek LM, Koopmans MP, de Jager CJ, Wannet WJ, Bartelds AI, et al. A comparison of gastroenteritis in a general practice-based study and a community-based study. Epidemiol Infect. 2001 Dec;127(3):389-97.

75. Guandalini S ea. Childhood acute diarrhoea in Italy. Pediatric Research. 1988;24:414.

76. Albano F, Bruzzese E, Bella A, Cascio A, Titone L, Arista S, et al. Rotavirus and not age determines gastroenteritis severity in children: a hospital-based study. Eur J Pediatr. 2007 Mar;166(3):241-7.

77. Elliott EJ, Backhouse JA, Leach JW. Pre-admission management of acute gastroenteritis. J Paediatr Child Health. 1996 Feb;32(1):18-21.

78. O'Loughlin EV, Notaras E, McCullough C, Halliday J, Henry RL. Home-based management of children hospitalized with acute gastroenteritis. J Paediatr Child Health. 1995 Jun;31(3):189-91.

79. Small F, Alderdice F, McCusker C, Stevenson M, Stewart M. A prospective cohort study comparing hospital admission for gastro-enteritis with home management. Child Care Health Dev. 2005 Sep;31(5):555-62.

80. Canani RB, Cirillo P, Terrin G, Cesarano L, Spagnuolo MI, De Vincenzo A, et al. Probiotics for treatment of acute diarrhoea in children: randomised clinical trial of five different preparations. BMJ. 2007 Aug 18;335(7615):340.

81. AIFA. Dear doctor letter. Il Bollettino di informazione sui farmaci 2004;1/2004:18.

82. Salazar-Lindo E, Santisteban-Ponce J, Chea-Woo E, Gutierrez M. Racecadotril in the treatment of acute watery diarrhea in children. N Engl J Med. 2000 Aug 17;343(7):463-7.

Research Activities II: implementation and skill transfer

The second part of the work has been focused on applying the knowledge acquired to collaborate in other studies. In the next section the study presented in detail, is a multi-centric study on prevention on HIV in which the candidate has played a role of site coordinator and co-investigator. Others were studies in which the candidate had different roles, including discussing the study, and participating in interpretation of results, writing and editing the final paper. Principal investigator of the study, dr MacLaren, is associated

HIV prevention strategy: acceptability of male circumcision

Description of the study is available at the official study website:

http://cmstest.jcu.edu.au/phtmr_public80_apr/abc/research/JCUPRD1_060921.html

Key points are here summarized below:

Introduction:

In recent years, male circumcision has been reported as effective in reducing the risk of HIV infection in HIV seronegative men in 3 large randomized clinical trials in African countries (Kenya, Uganda, South Africa). In particular HIV transmission was reduced by 60% in the South African[8] study, 53% in Kenya[9] and up to 53% in Uganda[10] . Based on this evidence, WHO recommendations for HIV prevention in resources limited settings, included male circumcision of HIV seronegative men in cases of high endemicity, prevalent heterosexual transmission and if acceptable by the population[1112] . In addition, countries with endemic that could possibly benefit from including male circumcision in the prevention strategy of HIV are invited to assess the

[8] Auvert, B. et al. 2005. Randomized, Controlled Intervention Trial of Male Circumcision for Reduction of HIV Infection Risk: The ANRS 1265 Trial. *PLoS Medicine*. 2:1112-1122

[9] Bailey, R. et al. 2007 Male circumcision for HIV prevention in young men in Kisumu, Kenya: a randomised controlled trial. *The Lancet*. 369:643-656

[10] Gray, R. et al. 2007 Male circumcision for HIV prevention in men in Rakai, Uganda: A randomised trial. *The Lancet*. 369: 657-666.

[11] http://www.who.int/hiv/topics/malecircumcision/en/

[12] UNAIDS & WHO 2007 *New Data on Male Circumcision and HIV Prevention: Policy and Programme Implications*. Geneva: UNAIDS.

acceptability and feasibility of the method. Previous studies on acceptability have been done in African countries; in a review of 13 of these studies, reported overall, results are of majority of men willing to be circumcised, if this is preventive for HIV, and of women to have partners and sons circumcised.

Papua New Guinea is a country with about 6,000,000 people, where HIV prevalence is roughly 1.5% and mostly via heterosexual transmission. Recent estimates of 76,000 people living with HIV are expected to rapidly increase up to hundred of thousands in case preventive interventions are not scaled up.

Characteristics of the epidemic in the country would make it a potential place to benefit MC as part of the prevention strategy, but acceptability and feasibility of the intervention in the country is not known. In addition, while MC was in the research priority agenda of the National AIDS Council.

This research project, founded by the Australian National Health and Medical Research Council (NHMRC; grant 601003) had the aim to investigate acceptability and feasibility of MC as part of the prevention of HIV in 4 sites in the country (PNG). The research aimed to document the current existing practices on MC, the social, cultural and religious factors impacting the acceptability of MC.

Aims:

Specific objectives of the study are:

1. To describe the male genital cuts occurring already in the population
2. To analyze the association of cultural religious, and social aspects and the acceptability of MC
3. To assess the feasibility of MC a health care settings

Design:

The study is a cross-sectional survey and the plan was to assess the current cutting practices; the social, religious, cultural aspects related to MC, the acceptability of MC for men and women, and the feasibility given the health providers beliefs and possibilities.

The tolls to collect the above mentioned data are: self administered questionnaires, medical examination (of male participants only), focus groups and in depth interviews.

The study is taking place in 4 sites of the country, 2 universities and 2 rural sites, each enrolling about 300 participants for questionnaires and medical examinations (of whom about 30-40 for focus groups) and 20 for in-depth interviews, at each site.

Expected results and importance of the study:

The results are expected to be informative for the policy makers and stakeholders in the planning of national plans for reducing of HIV. The survey should capture the acceptability of MC procedure at different levels, including the female population and the health workers. As previously objected, fro the particularity of this prevention being applicable only to men, women could be disadvantaged by the implementation of this prevention method, and therefore the opinion of women on the consequences and acceptability of MC are one of the important expected results of the study. In addition, the health care system, already spread thin in human and material resources, could be a significant obstacle to apply the method, even if found acceptable by the population, therefore the information collected at the Health System level are also fundamental for planning the next steps in the strategy for the national council.

In the context of this study, the candidate participated in the following activities:

At the central level, with the PI and other co-investigators:

Discussion and finalization of the research proposal, planning of data collection and analysis, preparation of organization of data collection tools (questionnaires and interviews templates)

At the site level, direct responsible for:

Selection, training and coordination of the research workers at the site; official relationships with local authorities and stakeholders for study support; information on the study in the university and local community ; coordination of activities to establish the study site and to organize the data collection, entering and managing.

The training of the research workers planned and conducted by the candidate, have included: principles of HIV epidemiology and medicine, and surveys technique, ethical requirements for conducting research.

Current status of the study: ongoing, at the level of data collection. The candidate is expected to collaborate in the analysis of data, discussion and interpretation of the results and writing of the final paper/s over the next months.

The list of principal investigators and other co-investigators for this study is as follow:

Principal investigator dr David MacLaren; co-investigators prof John McBride, dr Mike Wood, Ass. Prof. Alan Clough, dr. Lester Ross, dr Joseph Orathinkal.

Collaboration in other studies

Other preliminary experiences were started during the program to create opportunities for sharing research skills and assessing the needs of establishing an expertise within clinical realities, in different settings.

Few examples are here described.

Collaboration in the data discussion and interpretation, part of data analysis and paper drafting and writing, with a collaborative multicenter clinical trial, promoted by the Department of Nephrology of the Pediatric Department (University of Padova). The trial compared 3 different ways of preventing recurrent urinary infections in children and resulted in a publication in a peer reviewed journal (Pediatrics).

In a University of a low income country (DWU University, in Madang PNG), during last year, the candidate planned, organized and gave course for students in the Bachelor of Rural Health. The first course (45 hours) was on epidemiology, focused on how data can be collected at a primary level in order to be utilized for epidemiological information on disease occurrence, management and outcome of the diseases, with few elements of data analysis. The second course (45hours) was more specifically on research methodology. Focus was given to different studies designs and their advantages and disadvantages. In particular, students have been carried through example of how simple studies could be organized to answer a research question arising from what seen in the everyday patients' care. The course included also a substantial section on clinical trials, and emphasis on ethical issues in conducting research.

With the aim to introduce and promote the concept of evidence based science, and in collaboration with University of Melbourne (the Nossal Institute for Global Health)[13] the candidate co-authored a 4 days workshop on literature review methods (Madang, 22-25 March 2009). Main topics taught were use of pubmed for scientific literature and critical appraisal of papers. The target were lecturers of the faculty of health science, and a total of 16 participated. The training had two main objectives. The first was to make participants feeling confident in using in their everyday activities

[13] http://www.ni.unimelb.edu.au/

(either of medical practice or teaching) some of the methods used in research to look for evidence. The second to stimulate some of starting works of systematic literature review in collaboration of the University of Melbourne. In both cases, the final aim was to improve the quality of data searching in order to have up to date evidence based learning and teaching material.

Finally, both in the reality of University of Padova and in the novel DWU University, the candidate has, along the year, participated and promote collaboration with physicians who were conducting or approaching clinical studies. Some of the activities performed included: reviewing and sharing ideas and opinions on the methods applied in writing research proposals, preparing submissions to local or National Ethic committees, discussing analysis plans, interpreting the results of analysis previously performed, finding effective ways to present quantitative and qualitative research to peers in internal publications or international symposia.

Conclusion

Through this doctoral program the candidate could learn important skills in research methodology, building on previous medical experience in the field on infectious diseases and HIV in particular, but gaining also global perspectives. The interest for clinical research arisen during previous work appointments could be strengthen and the ability to perform it in real life research situation tested. As demonstrated by the research skills acquired and applied in the research works presented here, the candidate has reached the objectives of the doctoral program.

In particular the experience of formal theoretical learning in courses of biostatistics and epidemiology, has been fundamental, as previously lacking in her background medical education. Through the application of novel learnt skills (for example in data analysis) was possible to better comprehend the challenges and solutions of problems currently encountered in realization of clinical studies. As the application has always been performed in collaboration of experienced researchers, the candidate could benefit from exchanges with them, being continuously learning and studying in order to be able to be active part of the research projects.

The technical knowledge acquired includes conducting literature reviews, planning analysis of an observational study, analyzing data on the basis of a plan, using the statistic software (Stata and SAS) to perform analysis, collaborating with other researchers in discussing and interpreting the results obtained and writing publications (papers, posters and abstracts). In addition, collaboration with different research teams strengthened the ability to adapt to different needs for research and to English to exchange with different stakeholders associated with clinical research.

In conclusion, the gain in knowledge and experience in the application of epidemiology and biostatistics are important, and were performed in a selection of key settings, contributing to a versatile baggage of experience, fundamental to the realization of future clinical research. In addition, the candidate's previous specialization and experience in HIV and infectious diseases was enhanced and applied to global health. The next steps are the transferring of these capacities in settings were clinical care is practiced but little is available to conduct spontaneous research. In many clinical realities, there is an unutilized potential for observational research that could enhance the quality of care through the evidence gathered and through the diffusion of a quality assurance mentality that can be toughed through application of well conducted research. The aim of future work will be to facilitate and promote research in an array of clinical realities, with variable level of resources available. In this situation what has been acquired during this doctorate would be essential in: planning and establishments of observational cohort, planning and conduction of

cross-sectional or intervention studies, assist with data analysis and interpretation, collaborate in literature reviews and production of peer-reviewed papers.

Publications

Papers

- Fregonese F, Collins I, Jourdain G, et al. Survival at 5 years of HIV-infected adults starting highly active antiretroviral therapy (HAART) in Thailand: risk factors for early and long-term mortality. *Submitted to Clinial Infectious Disease.*
- Fregonese F, Wool PS, Girotto S et al, Epidemiology and management of acute gastroenteritis (AGE) in paediatric population, an observational retrospective study. *Submitted to European Journal of Pediatrics*
- Jourdain G, Wagner TA, Ngo-Giang-Huong N, Sirirungsi W, Klinbuayaem V, Fregonese F, et al. per Program for HIV Prevention and Treatment (PHPT) Study Group. Association between detection of HIV-1 DNA resistance mutations by a sensitive assay at initiation of antiretroviral therapy and virologic failure. Clin Infect Dis. 2010 May 15;50(10):1397-404.
- Sturkenboom M, Soriano-Gabarro M, Picelli G, Scamarcia A, Fregonese F, Cantarutti L, Franco E and Giaquinto C. Incidence and Outcomes of Acute Gastroenteritis in Italian Children . The Pediatric Infectious Disease Journal, Volume 27(1) Supplement, Jan 2008,pp S42-S47
- Giaquinto C, Fregonese F, et al. “Directions Delivered from Death's Door: 10 Years of Pediatric AIDS”, in: Zuniga JM, Whiteside A, Ghaziani A, Bartlett JG: “A Decade of HAART: Historical Perspectives and Future Directions”, Oxford University Press (2008)
- Montini G, Rigon L, Zucchetta P, Fregonese F, et al. ; IRIS Group. Prophylaxis after first febrile urinary tract infection in children? A multicenter, randomized, controlled, noninferiority trial. Pediatrics. 2008 Nov;122(5):1064-71.
- Giaquinto C, Morelli E, Fregonese F, et al. Current and future antiretroviral treatment options in paediatric HIV infection. Clin Drug Investig. 2008; 28(6):375-97. Review.
- Giaquinto C, Rampon O, Penazzato M, Fregonese F, et al. Nucleoside and nucleotide reverse transcriptase inhibitors in children. Clin Drug Invest 2007; 27 (8): 509-531

Abstracts

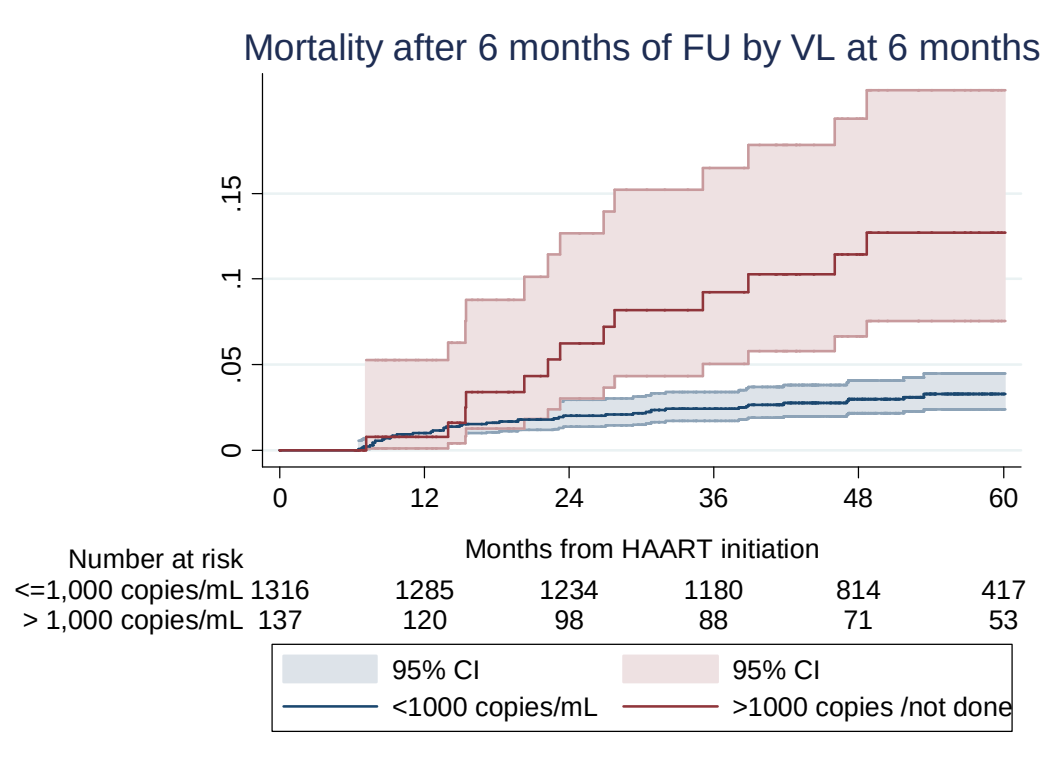
- Fregonese F, Collins I, Jourdain G et al. Long Term Survival of HIV-infected Adults Starting Highly Active Antiretroviral Therapy (HAART) in Thailand: Risk Factors for Early and Late Mortality. Poster Z-172, 18th Conference on Retrovirus and Opportunistic Infections, Boston, February 2011.

- Fregonese F, Yang H, Saeed S, Klein MB for the Canadian Co-Infection Cohort Study. Predictors of End Stage Liver Disease (ESLD) in patients with HIV /HCV Co-Infection Abstract presented in HIV cohort workshop, Lisbon March 2009
- MacLaren D., Tommbe R., Fregonese F. PNG – JCU collaboration to investigate the acceptability of male circumcision for HIV prevention in PNG. 60th Fulbright Symposium – Cairns, Queensland, August 2010.
- Jourdain G, Leurent B, Klinbuayaem V, Maynart M, Kantipong P, Techapornroong M, Banchongkit S, Fregonese F, et al. One year comparative hematological and renal safety of tenofovir + emtricitabine versus zidovudine +lamivudine in HIV-1 infected patients on first line nevirapine or efavirenz based therapy in Thailand, XVII International AIDS Conference Mexico City, August 2008, Abstract CDB0472

Appendices

Appendix 1

1. Estimates of mortality for viral load at 6 month
Considering missing VL as VL>1,000copies



Estimation of survival (after the first 6 months) for each year follow-up by viral load at 6 months:

Time	Beg. Total	Fail	Survivor Function	Std. Error	[95% Conf.	Int.]
vl <1000						
12	1287	13	0.9901	0.0027	0.9830	0.9942
18	1259	8	0.9839	0.0035	0.9754	0.9895
24	1235	5	0.9799	0.0039	0.9707	0.9863
36	1181	5	0.9759	0.0043	0.9659	0.9830
48	815	6	0.9703	0.0048	0.9592	0.9784
60	419	2	0.9673	0.0053	0.9553	0.9762
vl <1000 or not done						
12	121	1	0.9924	0.0075	0.9474	0.9989
18	106	3	0.9662	0.0166	0.9123	0.9872
24	99	3	0.9376	0.0229	0.8732	0.9698
36	89	3	0.9079	0.0279	0.8351	0.9495
48	72	2	0.8856	0.0314	0.8065	0.9337
60	54	1	0.8728	0.0334	0.7896	0.9246

Appendix 2A

Extracted from official website of The Canadian Co-infection Cohort at http://www.cocostudy.ca/index.html

"***The Canadian Co-infection Cohort Study*** *follows a group of HIV and hepatitis C (HCV) co-infected patients from 16 centers across Canada.*

The cohort was established in 2002 at three University centers in Quebec with infrastructure funding from the Fonds de la recherche en santé du Québec (FRSQ). In 2005 we received funding from the Canadian Institutes of Health Research (CIHR) to expand to an additional 13 sites across Canada.

The primary objective of this study is to determine the effect of highly active antiretroviral therapy (HAART) on liver disease progression in HIV-HCV co-infection. We have now enrolled 950 participants across Canada; a unique cohort similar to no other cohorts worldwide.

Participants are not required to take any special medications or change any of their behaviours, but are asked to complete a questionnaire every six months for five years. The questionnaires collect detailed information on demographics, drug and alcohol use, risk behaviours, smoking habits, quality of life measures as well as HIV and HCV treatment information. Blood samples are also collected and stored to perform viral, pathogenic and immunologic studies"

Appendix 2B

Poster presented at the 13th Workshop on HIV observational databases, Lisbon, March 2009.