IUT34-24 application 2015 Status: Funded

Application

Reasearch topic in Estonian	Infektsioonid langenud immuunsusega isikutel – eksperimendist kliinilisse praktikasse
Reasearch topic in English	Management of infections in the immunocompromised host – from bench to bedside
R&D institution	Tartu Ülikool
Research team leader (applicant)	Irja Lutsar
Start year	2015
End year	2020

Field of research, speciality and %

Field of research	CERCS specialty	Frascati Manual specialty	%
3. Health 3.1. Biomedicine	B460 Physical antropology	3.1. Basic medicine (anatomy, cytology, physiology, genetics, pharmacy, pharmacology, toxicology, immunology and immunohaematology, clinical chemistry, clinical microbiology, pathology)	50,0
3. Health 3.7. Clinical Medicine	B440 Human anatomy and morphology	3.2. Clinical medicine (anaesthesiology, paediatrics, obstetrics and gynaecology, internal medicine, surgery, dentistry, neurology, psychiatry, radiology, therapeutics, otorhinolaryngology, ophthalmology)	50,0

Application to expert comission	3. Health
Summary in Estonian	Projekti peamiseks eesmärgiks on parandada langenud immuunsusega haigete kliinilist

käsitlust. Planeeritud uuringud püüavad aru saada missugused faktorid mõjutavad antiretroviirusravi (ARV) saavate isikute immuunregulatsiooni. Teiseks püüame optimeerida antibiootikumide annustamist kriitilises seisundis olevatele haigetele. Uuringud hõlmavad järgmisi populatsioone: (1) sügavalt enneaegsed vastsündinud, (2) kriitilises seisundis septilised täiskasvanud ja (3) HIV-positiivsed isikud. Esimeses tööpaketis (WP-1) uuritakse erinevate antibiootikumide farmakokineetikat-dünaamikat leidmaks võimalusi individuaalseks doseerimiseks. WP-2s püütakse leida HIV reservuaare organismis ja hinnatakse missugused inimese ja viirusepoolsed geneetilised faktorid mõjutavad vastust ARV ravile. WP-3 töötab välja ja valideerib skooringsüsteemi hindamaks septiliste haigete gastrointestinaalse puudulikkuse riskifaktoreid. Projekti tulemusel peaks paranema ülalnimetatud haigete ravi ja prognoos.

Summary in English

The main aim is to improve the management of immunocompromised patients by better understanding of immune regulation in HIV infected patients receiving ARV therapy and characterising PK/PD properties of antibacterial agents in extreme conditions like prematurity and critical illness in adults. Three populations – (1) premature and critically ill neonates; (2) adults in critical condition with risk of opportunistic infection and gastrointestinal failure and (3) HIV positive subjects undergoing treatment are included. In WP-1 the PK/PD properties of various antibiotics are described with the ultimate goal to define personalised dosing recommendations. In WP-2 the latency reservoirs of HIV will be characterised and relationships between viral- and host genetic factors are described. In WP-3 a scoring system to define outcome risks in patients with gastrointestinal failure will be developed. Ultimately this project will improve management and outcome of the immunocompromised host.

Sum applied for 2015. year with overhead

272 000,00 EUR (4 255 875 EEK)

Additional financial resources will be channeled into the research topic

☐

Ethics Committee Decision

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Overview of R&D activities by host institution

Institutional relevance (To be filled in by institutional representative)	In the University of Tartu (UT) there are 15 ongoing targeted financing projects and institutional research funding projects in 2014 (2 of them ending in 2014) which are connected to the specialty Biomedicine (ETIS classification 3.1), total funding of this specialty in 2014 is 1420527 EUR, 10,8% of the same type of funding in UT. In UT there are 11 ongoing targeted financing projects and institutional research funding projects in 2014 (2 of them ending in 2014) which are connected to the specialty Clinical Medicine (ETIS classification 3.7). The total funding of this specialty amounts to 813702 EUR in 2014 corresponding to 6,2% of the same type of funding in UT. Both specialty mentioned above belong to the curriculum group of medicine which is the responsibility of UT. In 2009-2014, 74 PhD degrees have been conferred in these specialties. In 2014 the number of post-graduates is 189. Clinical medicine is a field where UT belongs to the 1% of the most cited in Thomson Reuters Essential Science Indicators.
National relevance (To be filled in by institutional representative)	The proposed project corresponds to the main objective of strategy of the Estonian Government for 2014-2020 “Knowledge-based Estonia”. This project is in good accordance with the priorities of National Health Plan 2009-2020: prevention, diagnostics and treatment of chronic diseases and malignant tumors which have the most significant impact on population health; development of personal medicine by evaluation and taking account of individual risk factors in planning prevention and intervention strategies. The project is closely related and connected with the Translational Medicine infrastructures, which is a part of “Estonian research infrastructures roadmap”. The proposal is in accordance with the University of Tartu Strategic Plan 2009-2015: in all its fields of teaching and research, the University of Tartu ensures a standard that is internationally recognized and the best in Estonia.
Information about related previously financed research topics (To be filled in by institutional representative)	Research topic is closely related to and is a continuation of the targeted financing project: “Infection and immunocompromised host - from mechanisms to translation into clinical practice ” (SF0180004s12, Irja Lutsar, 2012-2014). Overall funding in 2012-2014 was 349 080 EUR (in 2012 and 2013 116360 EUR per year). In 2013 there were 18 main participants in this targeted financing project. Expenditures on staff were made to the workload of 2,99 (FTE): 1,77 research staff; 1,22 operating personnel. Expenditures in 2013 were 99873,35 EUR. This sum was divided as follows: staff costs 65808,90 EUR; travel costs 4033,20 EUR; other costs 30031,25 EUR.

Justification of the research topic

General motivation for the proposed research topic, its role and importance in science, its general theoretical background, main objectives and their justification	This application unites three immunosuppressed populations – (1) premature and critically ill neonates admitted to neonatal intensive care units (NICU); (2) adults in intensive care unit (ICU) with risk of opportunistic infections and acute gastrointestinal injury (AGI) and (3) HIV positive subjects (mainly intravenous drug users - IDU). Although these populations are just a fraction of the immunocompromised hosts they all are relevant in the Estonian context where the numbers of premature babies and elderly in ICUs are increasing due to improved survival rates of the former and life expectancy of the latter. Furthermore the number of newly infected HIV positives in Estonia is still the highest in European Union. The main aim of this research project is to improve the management of immunocompromised patients by characterising pharmacokinetic/pharmacodynamic (PK/PD) properties of antibacterial agents in the extremes such as prematurity and/or critical illness and to understand in depth the immune regulation in HIV infected patients undergoing antiretroviral (ARV) therapy. From the practical point of view we aim to define and translate into practice the risk assessment tools for patients with AGI and for HIV-patients undergoing ARV therapy. The PK studies aim to define covariates associated with inadequate (excess or too low) antibiotic exposure and result in antibiotic dosing recommendations (individual dosing algorithms) for critically ill neonates and adults on renal replacement therapy (RRT). We believe that knowledge collected in patients with HIV infection could also be translated to patients with other forms of acquired immunodeficiency such as critical illness and immunosuppressive therapy. For improved reading the scientific background and specific aims will be presented under each work-package (WP) separately.
A detailed justification of the proposed research topic, working hypotheses, methods etc., the outline the work plan (if appropriate, including an explanation of how ethics requirements will be adhered to in the case of animal and human experiments)	WP-1 –PK/PD of antibiotics in critically ill neonates and adults Existing recommendations for dosing of antibiotics, mostly based on studies performed in adults without serious underlying conditions with moderate disease, have not proven adequate in severe disease and immunocompromised host [13, 70]. PK/PD target optimisation strategies, like prolonged or continuous infusion of beta-lactams have not demonstrated convincing clinical benefit [54]. Underlying condition, body size, disease severity and age affect volume of distribution (VD) and drug elimination significantly [3, 10, 43, 73, 74]. In the critically ill patients changes in physiology like altered protein binding, poor tissue penetration (perfusion) and fluctuations in VD and clearance lead to further variation in drug concentrations, compromising safety but also efficacy [17, 31, 58]. Over a third of adult critical care patients with creatinine clearance exceeding 130 ml/min will not achieve the minimum therapeutic target of 50% fT >MIC with current extended infusion of meropenem and piperacillin/tazobactam [6]. Therapeutic interventions such as RRT, extracorporeal membrane oxygenation, volume replacement/ dilution contribute to variability [20, 23]. Two- to ten-fold variations in plasma concentrations of various antibiotics (meropenem, piperacillin/tazobactam, vancomycin, ciprofloxacin) have been described in adults receiving RRT with no correlation between clearance and RRT settings [52, 69]. In addition, higher therapeutic targets are likely required for efficacy in the immunocompromised host and more resistant bugs often encountered in ICU [24, 31]. Subtherapeutic antibiotic doses may promote treatment failure or the selection of resistant pathogens [71]. The coexistence of subpopulations with decreased susceptibility increases the probability of these subpopulations surviving under inadequate antibiotic exposure, and eventually acquiring additional resistance mechanisms [51, 65]. At the same time PK/PD indices (drug exposure ensuring an optimal

effect) of beta-lactams are well known - the time during which the free drug concentration (unbound fraction) of the drug remains above the MIC being the dominant PKPD index associated with bacterial killing [31, 32]. Therefore the need for therapeutic drug monitoring (TDM) together with individual dosing has been increasingly recognised in their use. Among coagulase-negative staphylococci (CONS), the predominant pathogen causing neonatal late onset sepsis, altogether 75–90% of isolates are resistant to methicillin, a first-choice antibiotic against staphylococcal infections. A great majority are resistant to aminoglycosides, commonly used for the treatment of Gram-positive infections [30]. Increase in MIC values or heteroresistance against vancomycin, a last resource antibiotic, has also been described and such endemic clones detected [9, 44, 64]. In our recent study 49% of neonatal S. epidermidis isolates were found to be heteroresistant to vancomycin [59]. The contribution of heteroresistance to the dissemination of S. epidermidis clones in NICU has been demonstrated once, but its role together with other virulence factors in epidemiologic success as well as treatment outcomes has been rarely evaluated [4, 9, 78]. Despite all recent efforts the PK/PD studies in critically ill patients, especially in premature babies are still very rare and most guidance documents are based on the small studies, case reports or expert opinion making them prone to inappropriate recommendations. We hypothesize that existing antibiotic dosing recommendations fail to ensure optimal drug exposure in a significant proportion of the highly variable populations of critically ill neonates and adults. Beta-lactam TDM together with optimised PK/PD index based individual dosing will allow improved therapeutic target attainment. Better recognition of the mechanisms behind the heteroresistance of CONS together with in vitro PK/PD modelling will allow developing optimal dosing regimens of vancomycin in preterm neonates. The ultimate aim is to personalise dosing recommendations of antibiotics for critically ill adults and neonates to eventually improve their clinical outcome. The aim: 1. To describe the PK/PD of vancomycin and selected beta-lactams in neonatal and adult intensive care patients, including during high volume RRT; 2. To test bed-side TDM methodology for the assessment of beta-lactam antibiotic free concentrations to inform individual dosing in patients with elimination organ failure and/or infected with intermediately resistant microorganisms; 3. To identify difficult to predict covariates (i.e. disease severity, fluid status etc) significantly affecting the PK profile of beta-lactams and by modelling technique to define algorithms for individual dosing in clinical situations with the highest PK variability; 4. To identify a therapeutic drug monitoring strategy for beta-lactams and vancomycin ensuring maximum efficacy with minimal toxicity in the critically ill and develop computer based algorithms for individual dosing recommendations 5. To describe the epidemiology of vancomycin heteroresistance in neonatal CONS isolates and by in vitro PK/PD modelling to optimise vancomycin therapeutic targets for heteroresistant CONS infection in neonates 6. To improve patient outcomes in intensive care by optimised therapeutic targets and individual dosing of frequently used antibiotics. The first set of studies will continue our previous work, focusing on the PK/PD of antibiotics in the immunocompromised populations [25, 26, 36, 38, 66]. Previously developed multiple drug measurement methodology in small volume samples will be applied. In adults, we will continue PK studies in ICU patients on RRT [66]. In neonates, we have recently demonstrated that according to currently available TDM based PK model for vancomycin [1] and the MIC distribution of neonatal isolates the PD target of AUC/MIC of 400 will be achieved for >80% of patients only by doubling the current dosing regimen [39, 67]. With intermittent bolus dosing this will result in trough concentrations exceeding 20

ng/ml for those with gestational age < 28 weeks, rising clearly safety concerns [8]. The following studies will be performed. A detailed description of PK studies is given elsewhere. Study 1 - a single dose rich sampling PK study of piperacillin/tazobactam in 12 adult ICU patients receiving high volume hemofiltration (HVHDF). PK/PD modelling approach will be applied to define optimal dosing for various HVHDF regimens. Study 2 - a steady state semi-rich (4-5 samples within dosing interval) sampling PK study of ampicillin and gentamicin in 20 neonates of various gestational ages. Study 3 - a steady state semi-rich sampling PK study of vancomycin in 10-15 critically ill neonates to inform PK/PD modelling of vancomycin time-concentration curve and clearance. The second set of studies is devoted to the development of bedside TDM. The HPLC-MS-MS methods of drug concentration measurement developed in our previous research [25, 38] will be employed for commonly used beta-lactams (meropenem, piperacillin/tazobactam) in septic neonates and adults. Recent clinical trials utilizing strategies that optimize drug exposure demonstrate superior surrogate outcomes but a mortality benefit is still uncertain [53, 72]. Individual dosing algorithms will be suggested and related to clinical efficacy and safety. Total drug concentrations will be compared to free fraction of drug as measured by a bed-side methodology customised in an FP7 funded research project Mon4Stat. The “best-in-class” population based PKPD targets and models from literature and our previous research [25-27, 36, 38] will be collected and refined by integrating the information gained from clinical studies using statistical methods (Empirical Bayesian Estimation). The following studies are planned Study 4 - a prospective two-centre study customizing a new bed-side methodology for monitoring free beta-lactam concentrations in neonatal sepsis with the aim to develop an approach for managing the deviation between experimentally observed beta-lactam blood levels and PK/PD targets. Subsequently, the observed deviations will be translated into individual dosage corrections. Study 5 - a prospective TDM study of meropenem and piperacillin/tazobactam free and total beta-lactam concentrations in adult ICU patients with sepsis with subsequent individual dose adjustment based on optimised PK/PD targets A TDM database will be developed for investigation of the covariates of beta-lactam dosing in ICU patients. Study 6 - a prospective two-centre study of the impact of ICU environment and breast milk feeding on neonatal CONS colonisation, followed by in vitro PK/PD modelling studies of the effect of variable vancomycin time-concentration profiles on the time-killing of vancomycin heteroresistant vs sensitive CONS isolates. Sample size and statistical considerations No formal sample size calculation will be performed for the PK studies; as common in rich or semi-rich sampling PK studies each treatment regimen will be given to 9 to 15 subjects (studies 1-3). Only one dose will be evaluated on the premises that the PK profile of beta-lactams is usually linear. The second package of studies includes observational studies with estimated sample sizes based on available patient populations. Up to 100 patients with sepsis and pneumonia are being treated in the study site every year. The colonisation study as a pilot study will include 50 pairs of mother/preterm infant (GA < 32 weeks and BW < 1500 g); 10 pairs of mother/late preterm neonate (GA 32-36 weeks and BW 1500-2500 g); 20 pairs of mother/term neonate (GA >37 weeks and BW >2500 g). Detailed protocol for study 4 is under development. WP2 - Host and pathogen relations in HIV infection Modern HAART effectively suppresses HIV replication below the detection limits of clinical assays. However, despite prolonged successful treatment several challenges remain including persistent low level viremia, low number of latently infected cells and chronic immune activation. Despite of numerous studies the mechanisms behind the progressive loss of CD4+ T cells during the

course of HIV infection is still unclear. Studies suggest that chronic immune exhaustion is likely the result of complicated interactions between several factors, the most important of which is the formation of latent HIV reservoirs and disruption of signalling and gene expression pathways responsible for T cell homeostasis. Recently it has been shown that replication competent latent reservoirs remain active for several years under successful ARV treatment despite of very low number of latently infected peripheral blood monocytic cells (PBMCs) [12, 56]. In addition to latent reservoirs, studies suggest the persistence of ongoing viral replication under optimal ARV therapy, evidenced by unstable unintegrated viral DNA in PBMCs and viral genomic RNA blips in the blood [7, 12]. The immune activation is further complicated by the leakage of bacterial products from the gut (due to the loss of Th17 cells), low level presentation of antigens of endogenous pathogens (e.g. EBV, CMV) or aforementioned low level persistence and replication of HIV. As a result these processes evoke disruptions of T-cells signalling and homeostasis pathways further modulated by genetic and epigenetic factors. We and others have previously demonstrated the role of IL-2-CCR5-STAT5 signalling pathway in the pathogenesis of HIV infection and suggest that its modulation at genetic and epigenetic level in T cells is likely one of the key factors of the immune activation [5, 21]. Genomes wide as well as locus specific genetic studies have found HLA, interleukins, chemokines, especially CCR5 and its ligands, to play an important role in this process. However, despite extensive investigations genetic factors describe only a fraction of heritability in infectious diseases as well as susceptibility to and progression of HIV infection [35]. Recent studies indicate that the important pathways and regulatory mechanisms of certain diseases could also be regulated by epigenetic mechanisms at miRNA, gene expression or chromatin accessibility level. The majority of HIV pathogenesis associated signalling pathways have not been described at the epigenetic level. We hypothesize that the driver of productive infection including the source of low level blood viremia originates from certain anatomical locations and cell types, especially from some regions of the brain. These compartments may be influenced by interindividual differences, viral genetic polymorphisms and the type of HAART. Second we hypothesize that immune activation is associated with the expression (RNA-seq), accessibility (formaldehyde-assisted isolation of regulatory elements followed by high-throughput (HTP) sequencing – FAIRE-seq) and transcription factors occupancy (chromatin immunoprecipitation followed by HTP sequencing – ChIP-seq) in the IL-2-CCR5-STAT5 pathway genetic regions. Two studies will be performed with the specific aims: 1. To determine HIV phylodynamics in different tissues and cell types 2. To determine the genetic and epigenetic differences in signalling pathways associated with immune activation prior to and during the HAART Study 1 – HIV-1 phylodynamic study We enrol about 20 HIV-positive subjects undergoing routine forensic autopsy (common practice in Estonia) due to violent death including drug overdose. There are about 120 such deaths annually, half of them registered in HIV-positive subjects. In about a third of the cases an autopsy will be performed within 48 hours after death and these will be suitable for the study. Subject's history will be extracted from the E-HIV database. At autopsy the samples will be collected from about 30 different locations relevant to HIV replication (e.g. numerous parts of digestive system, lymph nodes and brain) [15, 16, 57, 63, 75]. Cells will be separated from tissues using collagenases treatment, counted and sorted using antibody-staining. The number of HIV infected cells will be determined using p24 antibodies or limiting dilution PCR [77]. Different forms of viral RNA and DNA (plasma and cell viral genomic RNA, proviral and circular viral DNA) will be extracted and

sequenced (whole genome sequencing in four subgenomic regions) using Illumina HTP sequencing (at least 1000x overlap). Sequences will be used to construct the HIV viral diversity and phylodynamic relations between sequences extracted from different tissues, cell types and viral genomic forms. Study 2 - Genetic and epigenetic correlates of the HIV induced immune exhaustion during ARV therapy In Estonia about 300 patients will start ARV treatment yearly. The study will include about 280 patients initiating ARV therapy either with 2NRTI [ABC+3TC/ TDF+FTC]) plus NNRTI [EFV] or plus PI [LPV/DRV/fAMP] based regimen using 1:1 randomisation scheme. Clinical data and blood samples will be collected as defined in E-HIV database. Study flowchart is presented on Figure 1. The immune activation of HIV associated T-cells will be measured by flow cytometry (CD3, CD4, CD8, CCR7, CD45RA, CD45RO, CCR5, CD38, HLA-DR) in all subjects. The host genotyping in targeted regions (exomes and regulatory regions of HIV related genes e.g. CCR5, CCL3L1, TLR3, IL-2, IL-10) and HIV-1 genotyping including the detection of viral tropism and DRMs will be performed as described elsewhere [2, 22]. The concentrations of ARVs will be measured to exclude non-compliant patients. A substudy of host epigenetic factors will compare 20 immunological super-responders (SR) with 20 nonresponders (NR) selected from 280 patients included into main study and classified by the CD4+ T-cell recovery rate during 2 years of potentially efficacious HAART. The 3 HTP methodologies using Illumina HiSeq will be used: • mRNA expression (mRNA-seq) analysis from CD4+ and CD8+ T-cells including naïve and memory (central, effector) subpopulations. • ChIP-seq (STAT5, H3K4me3; from CD4+ and CD8+ whole T-cells). The selection criteria for the two ChIP-seq targets rely on the involvement of transcription factor STAT5 in HIV disease related IL-2-CCR5-STAT5 signalling pathway [5]. H3K4me3 ChIP-seq is able to determine the differences in the accessibility of regulatory regions in promoter/enhancer region which could differentiate SR and NR gene regulatory profiles [18]. • FAIRE-seq (from CD4+ and CD8+ whole T-cells) is able to determine the intra and extragenic region openness differences in SR and NR allowing to find HIV disease associated regulatory regions also outside the known genetic loci [60]. The selection of H3K4me3 ChIP-seq and FAIRE-seq is based on their discrete peaks (e.g. need smaller read numbers compared to other ChIP-seq markers) making them suitable for the population based stuies. The bioinformatic analysis of collected sequences in both studies of this WP will be conducted using Galaxy and TopHat HTP sequencing analysis software [14, 28], BioConductor (peak-calling and statistical analysis) and in-house written python and R scripts. The computation will be carried out in cooperation with the computing cluster of the University of Tartu. WP-3 – Acute gastrointestinal injury in critical illness We and others have demonstrated the importance of gastrointestinal (GI) tract in critically ill patients. Up to 59% of patients exhibit at least one GI symptom during their stay in ICU [48, 49], and development of GI symptoms and intra-abdominal hypertension (IAH) is associated with worse outcome [33, 34, 46, 50]. Therefore, further studies focusing on mechanisms and treatment possibilities of GI failure are urgently needed. The first set of studies will be undertaken to address different aspects of epidemiology and severity of IAH. It is known that IAH affects approximately a third of ICU patients. The IAH can be either primary or secondary depending on whether the underlying disease is of abdominal origin or not. The differences in epidemiology and outcome between primary and secondary IAH have been assessed only in one study previously [45]. Further, the relevance of abdominal perfusion pressure (APP, calculated as the difference between mean arterial pressure (MAP) and IAP) has been repeatedly proposed but not well demonstrated [29]. The second set of studies will be devoted to the development of a

valid tool for the assessment of GI function in ICU. We have participated in an international initiative, formulating consensus definition of AGI for ICU patients [47]. However, until now no study has prospectively evaluated these newly developed AGI criteria. It is well recognized that rather subjective application of the clinical definitions of GI symptoms may remain a main limitation in this setting. Recent studies have identified the following plasma parameters as possible markers for more subtle levels of GI dysfunction. • Intestinal Fatty-Acid Binding Protein (IFABP) is a plasma marker for early intestinal epithelial cell damage as it occurs during gut ischemia-reperfusion injury, chemical epithelial cell toxicity, and intestinal transplant rejection [11, 40]. • Citrulline is a semi-essential amino acid mainly synthesized by small bowel enterocytes by the conversion of glutamine [62]. Critically ill patients with shock have an acute reduction of functioning enterocyte mass and reduced gut citrulline synthesis, leading to a low plasma citrulline concentration [41]. • Ileal Lipid Binding Protein (ILBP), formely named Intestinal Bile Acid-Binding Protein (I-BABP) is the only physiologically relevant bile acid-binding protein in the cytosol of ileocytes. Stressful stimuli such as exercise-induced splanchnic hypoperfusion increase the plasma concentrations of ILBP [76]. • D-lactate has been suggested to be a better marker of splanchnic hypoperfusion than its L-isomer. D-Lactic acidosis in patients was originally described during short bowel syndrome [37]. Acute intestinal ischemia causes a rise in D-lactate levels in the systemic circulation [42, 61] and disturbances in its concentrations were an important determinant of outcome in critically ill patients [55]. The above listed parameters have been so far investigated mainly in acute intestinal ischemia [68], but not in the broader context of GI dysfunction or failure. If these biomarkers will demonstrate sufficient sensitivity and accuracy in characterising GI function, either alone or in combination with GI symptoms, it would enable us to standardize the assessment of GI function in ICU patients. This is an important prerequisite for treatment strategies devoted to diminishing the impact of acute GI injury in critical illness. We hypothesize that intestinal-specific plasma and urinary markers are good to excellent predictors of outcome in critically ill patients. The best prediction is achieved by combination of GI symptoms, IAP and plasma markers, and thereby we will develop a 5-graded score, which accurately reflects the GI function of patients' in intensive care. A clinical score developed based on the data from this study can be combined with existing SOFA score and thereby the predictive value of the SOFA score will be improved. We aim to describe the role of AGI in critical illness. In adults, we plan to implement the consensus definitions and score for characterisation of AGI. Further, we intend to clarify if intestinal specific plasma and urinary markers have additional value in the assessment of GI function in this clinical setting. The following clinical studies are planned: Study 1 is a retrospective analysis of to characterise the differences and epidemiology of primary and secondary IAH. Also, the associations between IAP, APP and outcome in different grades of IAH will be addressed. Study will be based on analysis of clinical database of the General ICU of Tartu University Hospital. The live database includes clinical data including GI symptoms and IAP of more than 1500 consecutive patients since 2004. Study 2 is a prospective, multicenter study on prognostic value of GI symptoms and intestinal-specific biomarkers in prediction of intensive care outcome. The study will be performed in two parts. Part A aims to prospectively evaluate the value of GI symptoms alone and in combination with IAP, and AGI grades, as defined by the ESICM Working Group on Abdominal Problems, in prediction of intensive care outcome. Part B aims to determine whether biochemical markers (plasma and urine) of intestinal injury are of additional prognostic value

compared to clinical GI symptoms and AGI grades. A total of 500 patients from 15 study sites, including Tartu University Hospital will be included. Sample size calculation based on earlier studies indicated that 428 patients should be analyzed to detect a 5% increase in the predictive capability between SOFA and Gastro-Intestinal Failure score (based on the AUC of the ROC curve of the SOFA score of 0.750 (SD 0.25)). Sample size calculation for outcome laboratory parameters is based upon pilot data in 30 critically ill patients with IFABP concentration in survivors of 115 (100) pg/mL vs 185 (185) pg/mL in non-survivors with a type I error of 5% and type II error of 10%. The number of patients needed to detect such a difference is 150. The exact study protocol is available on request. Briefly, consecutive adult patients admitted to the ICU will be monitored for GI symptoms, IAP and AGI grades. Of these 500 patients, 200 will be included in part B, for assessment of intestinal-specific markers. The blood and urine samples will be collected daily from the 1st until the 7th day in ICU. I-FABP, citrulline, ILBP, and D-lactate will be determined by well-validated methods in the laboratory of Maastricht University Medical Center. Admission parameters to be documented include age, gender, medical/surgical profile, diagnosis, APACHE II score, and SAPS II score. The following parameters will be assessed daily on days 1 to 7: ¯ GI signs and symptoms both as determined from previous ESICM endorsed AGI definitions and as real-time values where appropriate; ¯ The likely reasons for AGI (underlying pathology or putative mechanism); ¯ Feeding details and related medications such as laxatives and prokinetics; ¯ SOFA sub-scores; ¯ Mean and maximum IAP, APP in all patients with a bladder catheter. The primary outcome parameters are 28- and 90-days all-cause mortality. Secondary outcomes include ICU and hospital mortality, length of stay, and duration of mechanical ventilation. Univariate analysis of collected data will be used to identify independent risk factors for primary outcome. A stepwise approach will be used to test factors with a $p< 0.2$ in univariate analysis associated with mortality and morbidity in a multiple logistic regression analysis. Results will be used to calculate receiver operator curves (ROC) characteristic and formulate the options for the new AGI score. To find the cut-off points of concentrations of plasma and urinary intestinal-specific markers that most accurately discriminated patients with morbidity and/or mortality, ROC characteristics will be drawn by plotting sensitivity against 1-specificity for all thresholds. Standards for Reporting of Diagnostic Accuracy statement for reporting studies of diagnostic accuracy are used in this study. Ethics All studies are conducted following ethical principles in accordance with European and Estonian legislation. Patient's rights and dignity will prevail over that of science and society; any risks are to be balanced with benefit. Approval of ethics committee will be obtained for all studies and written consent will be signed by all study participants. Where the study subject is unable to sign such consent, it will be signed by next of kin (in critically ill adults and in the autopsy study) or by parents or guardians in neonates. In E-HIV database all patients have consented for post-mortem use of their data for research purposes. All efforts will be made to cause minimal distress and discomfort. With respect to blood sampling, no additional lines will be necessary as patients in the (N)ICU have vascular access in place. In neonatal studies investigators will ensure that trial related blood loss will not exceed 3% of total blood volume as recommended by the respective EMA guideline. This amount has been shown to be safely taken by us previously [19]. Whenever feasible we will use laboratory leftover samples for drug concentration measurements. The collection and use of private information generated during the project will be reduced to a minimum on a "need to use" basis to ensure patient safety while allowing interpretation of the results. All data will be reported anonymously.

Outline of the expected results and possible future developments, their importance for science, society and culture	In WP-1 we believe that identification of efficacy related parameters of antibiotics in adult and neonatal ICU patients will allow optimisation of therapeutic targets and implementation of beta-lactam TDM together with individual dose adjustment ensuring maximum efficacy with minimal toxicity as well as suppression of resistance for critically ill immunosuppressed patients. In HIV infection (WP-2) we will identify anatomical sites and cell types that contain persistent viruses. A more detailed understanding of the relationships between host's genetic variability, HIV infection pathways and their role in immune exhaustion during HAART will enable to develop treatment strategies for elimination of HIV reservoirs and ultimately to improve outcome. Studies of WP-3 are expected to improve and standardize the assessment of GI function in ICU patients. It is expected that one, or a combination of several tested parameters, can be used as a predictive tool for GI dysfunction in the critically ill. This would be a ground-breaking result, since so far no parameter of gastro-intestinal function as a singular item is an adequate predictor of outcome and the reliable scoring system for GI dysfunction does not exist.
The list of references, pictures and schemes	Fail_References Lutsar IUT.pdf Fail_Figure 1 IUT.pdf

Publications

Name
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Related industrial property items

Relationship between the chosen entries and the research topic

The proposal is a continuation of our research in managing infection of the critically ill immunocompromised host. In this proposal the methodology used in completed or ongoing studies will be used but new and more up to date methods will also be implemented. We have modified and introduced the semi-rich sampling methodology of neonatal PK studies as described by Padari et al. (2011) and have identified factors associated with the PK properties of meropenem (and likely also other beta-lactams) in extremely premature neonates. We have participated in the population PK analysis of voriconazole (Karlson et al. 2009) and will implement similar methodology to studies included to this proposal in WP-1. For WP-2 several methods (HIV sequencing, Taqman Allelic Discrimination assay for identifying polymorphisms in CCR5 gene etc.) have been developed/modified by our group earlier and are detailed in publications of Huik et al. (2010), Huik et al. (2014), Huik et al. 2014 and Avi et al. (2010). We have demonstrate that gastrointestinal tract symptoms in critically ill patients are common and that their presence is associated with poor outcome (Reintam Blaser et al. 2011) We have participated in international initiative, which formulated consensus definition of acute gastrointestinal failure for ICU patients (Reintam Blaser et al. 2012) and these criteria will be used in WP-3 study 2.

Research topic team leader and seenior research staff

Person	Degree	Current position	Position as researcher from 01.01. 2015	Position as lecturer from 01.01. 2015	Period	CV
Irja Lutsar	doktorikraad	Tartu Ülikool, Arstiteaduskond, Mikrobioloogia instituut, Meditsiinilise mikrobioloogia ja viroloogia õppetool; meditsiinilise mikrobioloogia ja viroloogia professor (1,00)		Professor (1,00)	1.01.2015-31.12.2020	ENG
Joel Starkopf	doktorikraad	Tartu Ülikool, Arstiteaduskond, Anestesioloogia ja intensiivravi kliinik; anestesioloogia ja intensiivravi professor (1,00), Tartu Ülikool, Arstiteaduskond, Anestesioloogia ja intensiivravi kliinik;		Professor (1,00)	1.01.2015-31.12.2020	ENG

Person	Degree	Current position	Position as researcher from 01.01. 2015	Position as lecturer from 01.01. 2015	Period	CV
		anestesioloogia ja intensiivravi professor (1,00)				
Tuuli Metsvaht	doktorikraad	SA Tartu Ülikooli Kliinikum; vanemarst õppejõud (0,75), SA Tartu Kiirabi; lastereanimobiili arst (0,20)	Senior Researcher (0,50)	Associate Professor (0,25)	1.01.2015-31.12.2020	ENG
Radko Avi	doktorikraad	Tartu Ülikool, Arstiteaduskond, Mikrobioloogia instituut, Meditsiinilise mikrobioloogia ja viroloogia õppetool; meditsiinilise viroloogia teadur (1,00)	Researcher (1,00)		1.01.2015-31.12.2020	ENG
Annika Reintam Blaser	doktorikraad	Tartu Ülikool, Arstiteaduskond, Anestesioloogia ja intensiivravi kliinik, Anestesioloogia ja intensiivravi õppetool; teadur (1,00)	Researcher (1,00)		1.01.2015-31.12.2020	ENG

Please describe how the top competencies of the research staff are related to achieving the goals of research

The research group was formed about 4 years ago and has concentrated on studies in immunocompromised host. It is a multidisciplinary team consisting of medical microbiologists (IL, RA), intensive care specialists (JS, ARB, TM), neonatologists (TM), paediatric pharmacologists (IL, TM) and molecular biologists (RA). More senior members of the team are internationally well recognised. The team leader IL has extensive experience in clinical research for more than 20 years and is internationally recognised researcher. Recently her main focus has been first on PK/PD studies of antimicrobials in neonates and second on studies with HIV infected subjects. Most senior members participate in more than one WP. The WP-1 is supervised by IL, JS and TM. All have been involved in supervising and conducting several PK/PD studies and have all required qualifications for performing and analysing such studies. WP-2 is led by IL and RA. RA is a researcher of younger generation with all qualifications and past experience for studies in molecular biology including evaluation of phylogenetics of HIV as well as host genetic factors in HIV infected subjects. WP-3 is led by JS and ARB, both experienced and internationally recognised scientists in the field of gastrointestinal failure in critically ill adults. Although each WP has its specific task there are several overlaps between different WPs and thus the knowledge acquired in one WP could be translated to another.

Scientometric information about research staff (2004-2014)

List of publications according to classification of publications in Estonian Research Information System (2004-2014)

Other personnel participating in the implementation of the research topic

Person	Academic degree	Institution and occupation	Start Date	End Date	Graduate Student
Kersti Oselin	Doctor's Degree	SA Tartu Ülikooli Kliinikum; arst-resident onkoloogias (1,00), SA Tartu Ülikooli Kliinikum; Teadur (0,25)	1.01.2015	31.12.2020	
Kristi Huik	Master's Degree	Tartu Ülikool, Arstiteaduskond, Mikrobioloogia instituut; laborant (1,00), Tartu Ülikool, Arstiteaduskond, Mikrobioloogia instituut, Meditsiinilise mikrobioloogia ja viroloogia õppetool; meditsiinilise mikrobioloogia nooremteadur (1,00)	1.01.2015	31.12.2020	PhD Student
Eveli Kallas	Master's Degree	Tartu Ülikool, Arstiteaduskond, Mikrobioloogia instituut; laborant (0,50)	1.01.2015	31.12.2020	PhD Student
Ene-Ly Jõgeda	Master's Degree		1.01.2015	31.12.2020	PhD Student
Piia Jõgi		SA Tartu Ülikooli Kliinikum, Tartu Ülikooli Kliinikumi Lastekliinik; arst-õppejõud (0,50)	1.01.2015	31.12.2020	PhD Student
Georgi Nellis		SA Tartu Ülikooli Kliinikum, Tartu Ülikooli Kliinikumi Lastekliinik; arst-õppejõud (0,50)	1.01.2015	31.12.2020	PhD Student
Kadri Tamme	Master's Degree	Tartu Ülikool, Arstiteaduskond, Anestesioloogia ja intensiivravi kliinik, Anestesioloogia ja intensiivravi õppetool; anestesioloogia ja intensiivravi vanemassistent (0,50)	1.01.2015	31.12.2020	PhD Student
Kaidi Telling	Master's Degree	Tartu Ülikool, Arstiteaduskond, Mikrobioloogia instituut, Meditsiinilise mikrobioloogia ja viroloogia õppetool; spetsialist (1,00)	1.01.2015	31.12.2020	PhD Student

Person	Academic degree	Institution and occupation	Start Date	End Date	Graduate Student
Pilleriin Soodla			1.01.2015	31.12.2020	PhD Student
Heli Rajasaar		Tartu Ülikool, Arstiteaduskond, Mikrobioloogia instituut; kliiniliste uuringute koordinaator (1,00)	1.01.2015	31.12.2020	
Juri Karjagin	Doctor's Degree	Tartu Ülikool, Arstiteaduskond, Anestesioloogia ja intensiivravi kliinik; anestesioloogia ja intensiivravi vanemassistent (0,20), Tartu Ülikool, Arstiteaduskond, Anestesioloogia ja intensiivravi kliinik, Anestesioloogia ja intensiivravi õppetool; anestesioloogia ja intensiivravi teadur (0,25)	1.01.2015	31.12.2020	
Jana Lass	Doctor's Degree	SA Tartu Ülikooli Kliinikum; kliiniline proviisor (1,00)	1.01.2015	31.12.2020	
Helgi Padari		SA Tartu Ülikooli Kliinikum, Tartu Ülikooli kliinikumi anestesioloogia ja intensiivravi kliinik; arst (0,75)	1.01.2015	31.12.2020	PhD Student
Hiie Soeorg		Tartu Ülikool, Arstiteaduskond, Mikrobioloogia instituut, Meditsiinilise mikrobioloogia ja viroloogia õppetool; laborant (0,60)	1.01.2015	31.12.2020	PhD Student
Merit Pauskar	Master's Degree	Tartu Ülikool, Arstiteaduskond, Mikrobioloogia instituut; laborant (1,00)	1.01.2015	31.12.2020	
Ülle Parm	Doctor's Degree	Tartu Ülikool, Arstiteaduskond, Mikrobioloogia instituut, Meditsiinilise mikrobioloogia ja viroloogia õppetool; Teadur (0,25), Tartu Tervishoiu Kõrgkool; õppejõud (0,75)	1.01.2015	31.12.2020	
Liivi Maddison		SA Tartu Ülikooli Kliinikum; arst-õppejõud (0,50)	1.01.2015	31.12.2020	PhD Student
Tõnis Karki	Doctor's Degree	Tartu Ülikool, Arstiteaduskond, Mikrobioloogia instituut, Meditsiinilise mikrobioloogia ja viroloogia õppetool; mikrobioloogia dotsent (0,50), Tartu Ülikool, Arstiteaduskond, Mikrobioloogia instituut, Meditsiinilise mikrobioloogia ja viroloogia õppetool; meditsiinilise mikrobioloogia vanemteadur (0,50)	1.01.2015	31.12.2020	
Maarja Hallik			1.01.2015	31.12.2020	PhD Student

Person	Academic degree	Institution and occupation	Start Date	End Date	Graduate Student
Karin Kipper	Doctor's Degree	Tartu Ülikool; Teadur (1,00)	1.01.2015	31.12.2020	
Karolin Toompere	Master's Degree	Tartu Ülikool, Arstiteaduskond, Tervishoiu instituut; analüütik (1,00)	1.01.2015	31.12.2020	
Inga Karu	Doctor's Degree	Tartu Ülikool, Arstiteaduskond, Anestesioloogia ja intensiivravi kliinik, Anestesioloogia ja intensiivravi õppetool; teadur (0,50)	1.01.2015	31.12.2020	
Triin Jakobson		SA Tartu Ülikooli Kliinikum; arst-õppejõud anestesioloogia erialal (1,00)	1.01.2015	31.12.2020	PhD Student

Comments on other personel participating in the implementation of the research topic

The other team members are 13 doctoral students at various stages of their studies, 7 researchers with PhD degree and 2 supporting members with master degree. Each researcher has a specific task. KO is a pharmacologist with main task of pharmacological analysis including population-PK modelling, KK is a chemist (mainly involved in measurement of antibiotic concentrations) and JL is a clinical pharmacist, all will participate mainly in WP-1. JK is an intensivist and will participate in WP-1 and WP-3. TK and ÜP are microbiologists and will participate in WP- 2 and WP-1. IK is an intensivist and will participate in WP-1 and WP-3. HR is a HIV database project leader and MP is a lab technician who will mainly participate in WP-2. Two doctoral students KH and LM will defend their thesis in the first part of 2014 and will join WP-2 and WP-3, respectively. KT is a statiscian involved in all WPs. Each WP has also involvement of doctoral students.

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