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The predictive value of cell-surface markers in infections in critically ill patients: protocol for an observational study (ImmuNe FailurE in Critical Therapy (INFECT) Study).

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Introduction
Critically ill patients are at high risk of nosocomial infections, with between 20 and 40% of patients admitted to ICU acquiring infections. These infections result in increased antibiotic use, and are associated with morbidity and mortality. Although critical illness is classically associated with hyperinflammation, the high rates of nosocomial infection argue for an important of effect of impaired immunity. Our group recently demonstrated that a combination of 3 measures of immune cell function (namely neutrophil CD88, monocyte HLA-DR and % regulatory T-cells) identified a patient population with 2.4-5 fold greater risk for susceptibility to nosocomial infections.

Methods and Analysis
This is a prospective, observational study to determine whether previously identified makers of susceptibility to nosocomial infection can be validated in a multi-centre population, as well as testing several novel markers which may improve the risk of nosocomial infection prediction. Blood samples from critically ill patients (those admitted to ICU for at least 48 hours and require mechanical ventilation alone or support of two or more organ systems) are taken and undergo whole blood staining for a range of immune cell surface markers. These samples undergo analysis on a standardised flow cytometry platform. Patients are followed up to determine whether they develop nosocomial infection. Infections need to meet strict pre-specified criteria based on international guidelines, where these criteria are not met an adjudication panel of experienced intensivists are asked to rule on presence of infection. Secondary outcomes will be death from severe infection (sepsis) and change in organ failure.

Ethics and Dissemination
Ethical approval including the involvement of adults lacking capacity has been obtained from respective English and Scottish Ethics Committees. Results will be disseminated through presentations at scientific meetings and publications in peer reviewed journals.

Registration

The study is registered on clincialtrials.gov with number NCT02186522

Strengths and Limitations

Strengths

- Multi-site study, so increasing the generalisability of results obtained.
- Multi-site nature has produced a program of flow cytometry standardisation which we believe to be both robust and reproducible and so set the scene for potential clinical use of these assays should they prove to be of value.
- We have taken steps to try to minimise variability in the diagnosis of infection, through the use of rigid criteria and consensus review of cases which do not meet these criteria.

Limitations

The weaknesses of this study are that it is observational, and thus will not be able to inform clinicians of what actions they should take in response to these results should clinically useable tests be developed.

Introduction

Critical illness increases the risk of nosocomial infection, with between 20 and 40% of patients admitted to ICU acquiring infections during their critical care stay (1,2), a rate that approaches that seen in hematopoietic stem cell transplantation (3). Provision of organ support requires the disruption of epithelial and mucosal barrier innate immune system protection through the placement of devices such as endo-tracheal tubes, urinary catheters and central venous catheters. These infections are often bacterial and are associated with increased antibiotic use. (1, 2). In addition to bacterial infections, critically ill patients are at risk of reactivation of latent viral infections (4). Therefore, it is thought that the combination of immune vulnerability and microbial colonization are responsible for the high rates of nosocomial infection seen in critically ill patients (1).

Critical illness resulting from trauma, sepsis and post surgical complications all have commonality in the innate and adaptive immune responses. (5,6) Many diseases that can precipitate the need for exogenous organ support and admission to intensive care are characterized by a profound systemic inflammatory response (7), with associated immune cell activation (8) and immune system-mediated organ damage (9). However it is now increasingly apparent that this over-exuberant inflammation is accompanied by an equally vigorous counter-regulatory anti-inflammatory response (5,10). The apparently maladaptive, complex immune dysfunction in critically ill patients manifests across a range of cellular actions and functions, involving both the innate and adaptive arms of the immune system (5,10-15). Defects have been noted in neutrophils (11), monocytes (12,17), T lymphocytes (10, 13-15) and splenic dendritic cells (10). The recent identification of elevated proportions of regulatory helper-T cells (Tregs) in sepsis (14,15) is in keeping with the supposition that much of the immunosuppression arises from the over-activation of counter-regulatory mechanisms. In human and experimental sepsis, Tregs mediate lymphocyte anergy and are associated with worse outcomes (15).

Rationale for the study

Although it seems plausible that the immune defects found in critical illness are associated with an increased risk of acquiring nosocomial infections, the concept that immune cell abnormalities always precede nosocomial infections, necessary for causality, is inconsistently reported in the literature (14,16,). Following our previous study (11), which demonstrated the benefits of combining measures of immune dysfunction on predicting nosocomial infection, there is a need to validate the markers in a new cohort. Undertaking this as a multi-centre study will help develop standardised flow cytometric measures and improve external validity of the markers that predict risk of nosocomial infection. If a selected set of immune abnormalites that could be modified with a single intervention are present together, then development of a test could stratify patients for risk of nosocomial infections for targeted interventions (17). Finally, identifying

multiple cell defects / markers will help design future trials of therapies and prophylactic measures to prevent nosocomial infections.

Hypotheses

1 Markers of neutrophil, monocyte and T-cell hypoactivity will predict those critically ill patients who are at risk of nosocomial infection.
2 These markers will have added predictive value when combined
3 Additional predictive ability will be achieved through examination of monocyte and Treg subsets and other cell surface markers of immune cell function.

Primary aims

1 To validate the ability of neutrophil CD88, monocyte HLA-DR and percentage of Tregs to predict those patients at risk of nosocomial infection
2 To determine the optimal way of combining these measures to risk stratify patients
3 To develop a clinically useable test

Secondary aims

1 To determine the relationship between the measures outlined above and risk of death from sepsis
2 To determine whether more detailed phenotyping of monocyte and Treg subsets provides additional value in predicting risk of nosocomial infection
3 To explore whether other cell surface measures of immune cell function and phenotype may predict nosocomial infection.

Methods and analysis

This protocol outlines a multi-centre, prospective observational study in which critically ill patients will be recruited and assessments made of immune cell surface phenotypic markers at multiple time points. Patients will be followed to determine outcomes of interest, the primary outcome being development of nosocomial infection.

Study population

The population will be drawn from 4 UK adult intensive care units, consisting of:
1 Royal Infirmary, Edinburgh (liver/general unit)
2 Western Infirmary, Edinburgh (neurosciences/general unit)
3 St Thomas's Hospital, London (cardiac/general unit)
4 Sunderland Royal Hospital (general unit)

Consecutive patients will be eligible if they are age >16 (Scotland) or >18 (England), receiving support of level 3 care (i.e. requiring invasive support of

respiratory system alone, or two or more other organ systems (haemofiltration, inotropes/vasopressors) and predicted to remain in ICU for at least 48 hours.

Exclusion criteria are not being expected to survive for a further 24 hours, known or suspected ICU-acquired infection at time of screening (non-ICU acquired nosocomial infection – i.e. non-ICU healthcare associated infection is not an exclusion), known inborn errors of immune function, immunosuppression (corticosteroids up to 400mg hydrocortisone equivalent daily dose permitted), HIV infection, hepatitis B and C infection, receiving Extra-Corporeal Membrane Oxygenation (ECMO), pregnancy and previously enrollment in the study.

Co-enrolment is permitted where the overall phlebotomy burden on patients is acceptable (<50ml at any one time point and total phlebotomy load of <150ml), where the co-enrolled study is deemed unlikely to affect the primary endpoint of the INFECT study and where a formal co-enrolment agreement is in place.

Sampling schedule

Blood samples for flow cytometric analysis of surface receptor expression will be taken on study enrolment, and then on day 2 after study enrolment, and at 48 hour intervals until day 12, a maximum of 7 samples per patient. Patients discharged from ICU within the 16 day study window will have a maximum of 2 further samples taken at 3-4 day intervals up to day 12 of study to minimize burden of venepuncture. Where possible these will be collected at the time of routine venepuncture for clinical sampling. Patients will be followed for the development of infection for 16 days (by this time the great majority of infections had been acquired in the original study and few patients were left in the ICU alive and without infection). All survivors who remain in hospital will be followed to this point including those who have left ICU.

Clinical and demographic data will be collected including age, sex, functional co-morbidity index, smoking status, physiological data (APACHE II score on admission, SOFA score at baseline, full blood count and differential white cell count) and clinical data (admission diagnosis, admission source, antibiotics, invasive devices present and duration). Similar clinical data will be collected at each sample time point. Hospital outcome (i.e. discharged alive, died or transferred to another hospital) will be also be collected.

Definition of infection

Infections will need to meet strict, pre-defined criteria (see appendix A below). and will be assessed by research staff blinded to the immune phenotyping data. Suspected infections which do not meet the criteria for confirmed infection will be reviewed 'off-line' by a panel of clinicians blinded to the immune phenotyping data using information from a prospectively collected pro-forma. The outcomes from this will be "highly likely" infection and "unlikely infection/colonisation" (colonisation being where microbes are grown in the absence of evidence for infection). The day infection is acquired will be defined as the day the sample which shows positive for microbiology was taken. In the case of sterile cultures where the clinician strongly suspects infection the day of infection will be

defined as the day strong clinical suspicion was raised. Therefore outcomes will be a) "confirmed", b) "probable infection" and c) "unlikely infection/colonisation".

Patients who are transferred to a non study hospital will have data included up until day of discharge and will be followed up via telephone contact from the recording unit to ascertain whether there were any confirmed or suspected infections in the days following transfer (up to 16 days post-study entry). Patients transferred from one participating site to another will remain in the study and have data and samples collected as per study protocol.

Flow cytometric standardisation and sample staining

All sites have standardised on the same platform, the FACS Canto II (Becton Dickinson Biosciences, San Jose, CA, USA - from here on BDB), for flow cytometric analysis of samples. Machines will be standardised by monthly matching of target values using a common batch of Cytometry Setup and Tracking (CS&T) beads (BDB), and daily internal quality control using CS&T beads.

Leukocyte cell surface staining will be conducted using antibodies supplied by BDB. All sites use antibodies from the same batch. Staining, data capture and storage will be conducted in accordance with a single study standard operating procedure.

Flow cytometry data will be held centrally. Final analysis will be conducted using a single analysis platform, VenturiOne (Applied Cytometry, Sheffield, UK).

Cellular immunophenotyping.

The primary measures are to validate our previous findings (11), namely: neutrophil CD88, monocyte HLA-DR, and percentage CD4 cells expressing the CD25+/CD127lo regulatory phenotype, using the cut-offs defined by our previous study (11).

Additional phenotypic measurements include: neutrophil and monocyte activation markers (including CD11b, CD66b, CD312) , sub-types of regulatory T-cells and Treg activation, frequency of monocyte sub-sets and subset HLA-DR expression, frequency of dendritic cell sub-types, expression of monocyte and lymphocyte PD1 and PDL1, frequency of granulocytic and Monocytic myeloid derived suppressor cells, and frequency of B-cell subtypes.

Sample size

In a cohort studied previously (11), 34% of patients were confirmed as having secondary sepsis. We therefore expect the panel of markers to perform well when predicting 25%-45% of patients to have secondary sepsis. Across a broad range of possible positive predictive value (PPV) performance (50%-90%), the 95% confidence interval width for the PPV would range from ±5.5% to ±15.9%,

indicating moderately precise estimation of the PPV. Using a similar rationale, we would expect the 95% confidence interval width for the negative predictive value (NPV) to range from ±4.3% to ±10.8%, indicating precise estimation of the NPV. A conservative estimate of a 50% PPV with a 35% rate of secondary sepsis would yield a 95% confidence interval of 39-61% in a cohort of 200 patients. We also intend to recruit 20 patients initially to confirm the reformulated flow cytometric tests match the performance of those from the derivation cohort (11) and thus we propose recruiting to a 220 patient cohort

Informed consent

Consent and assent procedures will be conducted under the relevant legislation; in England (Mental Capacity Act, 2005) or Scotland (Adults with Incapacity (Scotland) Act, 2000) for consent/assent of adults without capacity. In England assent is obtained, where possible, following discussion with the patient's next of kin (personal consultee). Where a personal consultee is unavailable assent is provided by a professional consultee, being a senior medical professional who is not in the research team. In circumstances where next of kin are unable to attend the ICU promptly, deferred consent procedures are used.

In Scotland the patient's relative or welfare attorney provides consent. If the patient's relative or welfare attorney are unable to attend the ICU, consent may be provided in a telephone conversation providing a second member of staff witnesses the discussion.

Patients that recover capacity will be approached to provide retrospective consent.

Safety of participants

The only safety concern is that of potential additional venepucture in patients, the risks of this are of minor harm (bruising). Post-ICU sampling (where indwelling lines are not present for blood sampling) is limited to a maximum of two samples.

Data analysis plan

A detailed statistical analysis plan will be finalised prior to the locking of the study database.

Studies will be conducted to ensure the flow cytometric readings of each marker are reproducible to demonstrate they can be a useful test. Inter- and intra-observer reliability studies will be conducted with three expert readers of the data. 50 data files will be read to determine inter-observer agreement, with 13 files for intra-observer agreement. After a preliminary reliability study, an optimisation stage will occur with expert meetings to further improve flow cytometric gating strategies in problematic markers to ensure we do not wrongly reject markers. A second reliability study will then be conducted using the same sample size to select markers taken forward to future stages of analysis.

The main analysis of the primary outcome will be an analysis of the PPV and NPV of immune dysfunctions in predicting nosocomial infection, as well as the predictive ability of combinations of immune dysfunction. The primary analysis will include both 'confirmed' and 'probable' infections as 'infections', with analysis by 'confirmed' infections only conducted as a sensitivity analysis. Sensitivity and specificity will also be determined. 95% confidence intervals will be calculated for all measures of predictive accuracy.

As with the derivation cohort (6), 'immune function/dysfunction' will be defined by the timepoint before first nosocomial infection occurs, censored for two days prior to infection.

As a secondary analysis a Cox model of time to acquisition of infection will be fitted, with the classification 'immune dysfunction' or 'no dysfunction' as one independent variable. The other independent variables will be potential clinical confounders identified in previous epidemiological work looking at risk factors for nosocomial infection (1,2).

The association of immune dysfunctions with the secondary outcome measures ICU outcome (lived/died) and death from sepsis (yes/no) will be assessed using the same methods as the main analysis of the primary outcome.

Ethics and dissemination

The study has been approved by the Scotland A research ethics committee (13/SS/0022) for Scottish sites and Warwick and Coventry research ethics committee (13/WM/0207) for English sites. Local research governance approval has been granted by the 3 NHS organisations covering the 4 sites where the study is being conducted.

Study management

The study is managed by the Edinburgh Clinical Trials Unit (ECTU) and the Edinburgh Critical Care Research Group (ECCRG) at the University of Edinburgh, Edinburgh, Scotland.

Sponsorship

The study is co-sponsored by University of Edinburgh and NHS Lothian.

Duration of study

The study is planned to run for 3 years. The expected end date for recruitment is January 31st 2016.

Results from the study will be reported according to the STARD guidelines for reporting diagnostic accuracy studies and disseminated via presentations at scientific meetings and publications in peer reviewed journals

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Registration

The study is registered on clincialtrials.gov with number NCT02186522

Authors contributions

Study conception (TW, NW, AB, ACM), obtaining funding (TW, NW, JK, AB, JS, ACM), development of flow cytometry techniques (JR, AW, AR, SL, TM, GH, ID), development of statistical analysis plan (TW, DD, CJW, ACM), protocol development (TW, DD, JA, AR, NW, JK, MSH, AB, JS, ID, ACM) manuscript drafting (DD, ACM). All authors have reviewed and approve the final version.

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Competing Interests

No, there are no competing interests

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Appendix A- definitions of infection

Any new infection occurring after 48 hours of ICU admission will be deemed 'ICU-acquired'. For consistency infections arising within 48 hours of ICU discharge will also deemed 'ICU-acquired'.

Infections will be defined prior to start of the study as follows, based on the Hospitals in Europe Link for Infection Control through Surveillance (HELICS) criteria.

a) Ventilator-associated pneumonia: Requires radiographic, clinical and microbiological criteria to be met:

i. Radiological criteria.
CXR or CT scan showing new infiltrates, or worsening infiltrates without evidence of pulmonary oedema, and either pyrexia of >38^{o}C or white cells >12000/mm^3 or <4000/ mm^3.

These must be combined with one or more clinical criteria.

ii. Clinical criteria.
Worsening oxygenation – any increase in FiO_2 to maintain PaO_2 target, or an increase in PEEP, frequency or tidal volume, proning or paralysis to facilitate ventilation.
OR
Relevant clinical chest findings – auscultatory finding of crepitations, crackles or decreased air entry.
OR
Increased/changed sputum – any increase in volume, presence of muco-purulent or muco-purulent-bloody sputum.

iii. Microbiological criteria.
The above radiological and clinical criteria must be combined with positive quantitative BAL culture of >10^{4}CFU/ml (of >10^{3}CFU/ml on protected specimen brush (PSB) sampling) or positive pleural fluid or pulmonary/pleural abscess culture.
Where the diagnosis of VAP has been suggested by mini-BAL, endotracheal aspirate or where growth is below the 10^4 CFU/ml threshold or without any positive microbiology, adjudication is required.

Hospital-acquired pneumonia (HAP), i.e. nosocomial pneumonia in non-mechanically ventilated patients (or patient in ICU for >48 hours but ventilated for <48 hours), requires the same fulfilment of criteria as VAP except that sputum cultures with heavy growth of a single organism constitute a confirmed infection.

b) Catheter-associated infections
Positive culture (where semi-quantitative cultures available >15CFU) from an indwelling vascular line combined with either
Local inflammation and pus (catheter-related infection (CRI)) or

Improvement of inflammatory markers within 48 hours of removal (CRI) or Culture of the same organism from a peripheral blood culture (catheter-related blood stream infection (CRBSI).

c) Blood stream infection
One positive culture of a typical pathogen, coupled with evidence of systemic inflammation (WCC >12,000/mm^3 or <4000/mm^3, temperature ≥ 38^{o}C).

d) Urinary tract infection
Growth of 2 or fewer organisms at ≥ 10^5 CFU/ml combined with evidence of systemic inflammation (WCC >12,000/mm^3 or <4000/mm^3, temp >38^{o}C or shock without another identifiable cause).

e) Soft-tissue or surgical site infection
Evidence of pus/inflammation at site of presumed infection combined with a positive culture.

f) *Infective diarrhoea*
Evidence of diarrhoea (3 or more loose stools in 24 hour period or use of a feacal collector) combined with culture positive for diarrhoeal organism or detection of enteropathogenic toxin.

g) *Intra-abdominal infection*
Evidence of intra-abdominal collection identified on surgical or radiological investigation, combined with positive culture from surgical specimen, needle aspirate or drain.

h) *Spontaneous bacterial peritonitis (SBP)*
Evidence of infected ascites (fluid with >250 neutrophils/mm^3, or abdominal pain and blood WCC >12,000/mm^3 or <4000/mm^3, temperature ≥ 38^{o}C in the presence of ascites without other source of infection) combined with positive ascitic fluid culture. Negative ascitic fluid culture in the presence of clinical criteria for SBP would be 'probable' infection.

i) *Sinusitis*
Evidence of facial sinus fluid collection on radiological (plain film, CT or ultrasound) or direct clinical observation of purulent discharge from a sinus combined with positive culture of fluid.

Infecting organisms may be bacterial, fungal or viral. Viral infections must be accompanied by clinical, radiological or histological evidence of tissue inflammation (e.g. herpes simplex stomatitis, Varicella pneumonitis, CMV colitis). Viral positivity without evidence of tissue inflammation would be classified as reactivation.

A 'confirmed' infection is one which meets the above criteria

Where an infection is suspected by the clinical team but does not meet the criteria above the case will require a proforma completed and details sent to

members of an expert consensus panel who are blinded to the immune cell data. The proforma will be reviewed by two members of the panel who will independently adjudicate the infection as either 'probable' or 'unlikely', in the event of the judgements being different a third panel member will be asked with the majority decision being recorded.

A 'probable' infection is where the panel clinician thinks there is, on the balance of probabilities, an infection present and would consider antibiotic treatment and/or source control if the patient's clinical condition merited it. This category may include positive microbial cultures. An example would be culture of a classically non-pathological organism (e.g. single cultures of coagulase negative cocci or diphtheroids) associated with clinical evidence of infection/systemic inflammation. Classically non-pathological organisms can be classified as 'confirmed' infections where there is strong evidence

An 'unlikely' infection is where the panel clinician thinks there is a low probability of infection and would not consider antibiotic treatment and/or source control. Although positive microbial cultures could be included in this, this would be culture of a classically non-pathological organism (e.g. single cultures of coagulase negative cocci or diphtheroids) without evidence of systemic inflammation/infection or mixed growth of commensal organisms.

Systemic evidence of infection would require the presence of SIRS – specifically 2 or more of the following heart rate>90 beats per minute, WCC>12/mm^3 or <4/mm^3 or >10% band types, respiratory rate >20 breaths per minute or mechanical ventilation, and temperature of >38°C or <36°C. Additional evidence to consider would include reports of large numbers of neutrophils on sample microscopy, and clinical examination findings of pus or inflamed tissue.

'Unlikely' infection combined with a positive microbial culture would constitute colonisation.

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	21a	Distribution of severity of disease in those with the target condition	Na
	21b	Distribution of alternative diagnoses in those without the target condition	Na
	22	Time interval and any clinical interventions between index test and reference standard	Na
Test results	**23**	Cross tabulation of the index test results (or their distribution) by the results of the reference standard	Na
	24	Estimates of diagnostic accuracy and their precision (such as 95% confidence intervals)	Na
	25	Any adverse events from performing the index test or the reference standard	Na
DISCUSSION			
	26	Study limitations, including sources of potential bias, statistical uncertainty, and generalisability	Na
	27	Implications for practice, including the intended use and clinical role of the index test	Na
OTHER INFORMATION			
	28	Registration number and name of registry	9
	29	Where the full study protocol can be accessed	9
	30	Sources of funding and other support; role of funders	9

STARD 2015

AIM

STARD stands for "Standards for Reporting Diagnostic accuracy studies". This list of items was developed to contribute to the completeness and transparency of reporting of diagnostic accuracy studies. Authors can use the list to write informative study reports. Editors and peer-reviewers can use it to evaluate whether the information has been included in manuscripts submitted for publication.

EXPLANATION

A **diagnostic accuracy study** evaluates the ability of one or more medical tests to correctly classify study participants as having a **target condition.** This can be a disease, a disease stage, response or benefit from therapy, or an event or condition in the future. A medical test can be an imaging procedure, a laboratory test, elements from history and physical examination, a combination of these, or any other method for collecting information about the current health status of a patient.

The test whose accuracy is evaluated is called **index test.** A study can evaluate the accuracy of one or more index tests. Evaluating the ability of a medical test to correctly classify patients is typically done by comparing the distribution of the index test results with those of the **reference standard**. The reference standard is the best available method for establishing the presence or absence of the target condition. An accuracy study can rely on one or more reference standards.

If test results are categorized as either positive or negative, the cross tabulation of the index test results against those of the reference standard can be used to estimate the **sensitivity** of the index test (the proportion of participants *with* the target condition who have a positive index test), and its **specificity** (the proportion *without* the target condition who have a negative index test). From this cross tabulation (sometimes referred to as the contingency or "2x2" table), several other accuracy statistics can be estimated, such as the positive and negative **predictive values** of the test. Confidence intervals around estimates of accuracy can then be calculated to quantify the statistical **precision** of the measurements.

If the index test results can take more than two values, categorization of test results as positive or negative requires a **test positivity cut-off**. When multiple such cut-offs can be defined, authors can report a receiver operating characteristic (ROC) curve which graphically represents the combination of sensitivity and specificity for each possible test positivity cut-off. The **area under the ROC curve** informs in a single numerical value about the overall diagnostic accuracy of the index test.

The **intended use** of a medical test can be diagnosis, screening, staging, monitoring, surveillance, prediction or prognosis. The **clinical role** of a test explains its position relative to existing tests in the clinical pathway. A replacement test, for example, replaces an existing test. A triage test is used before an existing test; an add-on test is used after an existing test.

Besides diagnostic accuracy, several other outcomes and statistics may be relevant in the evaluation of medical tests. Medical tests can also be used to classify patients for purposes other than diagnosis, such as staging or prognosis. The STARD list was not explicitly developed for these other outcomes, statistics, and study types, although most STARD items would still apply.

DEVELOPMENT

This STARD list was released in 2015. The 30 items were identified by an international expert group of methodologists, researchers, and editors. The guiding principle in the development of STARD was to select items that, when reported, would help readers to judge the potential for bias in the study, to appraise the applicability of the study findings and the validity of conclusions and recommendations. The list represents an update of the first version, which was published in 2003.

More information can be found on http://www.equator-network.org/reporting-guidelines/stard.

BMJ Open

The predictive value of cell-surface markers in infections in critically ill patients: protocol for an observational study (ImmuNe FailurE in Critical Therapy (INFECT) Study).

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The predictive value of cell-surface markers in infections in critically ill patients: protocol for an observational study (ImmuNe FailurE in Critical Therapy (INFECT) Study).

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Introduction
Critically ill patients are at high risk of nosocomial infections, with between 20 and 40% of patients admitted to ICU acquiring infections. These infections result in increased antibiotic use, and are associated with morbidity and mortality. Although critical illness is classically associated with hyperinflammation, the high rates of nosocomial infection argue for an important of effect of impaired immunity. Our group recently demonstrated that a combination of 3 measures of immune cell function (namely neutrophil CD88, monocyte HLA-DR and % regulatory T-cells) identified a patient population with 2.4-5 fold greater risk for susceptibility to nosocomial infections.

Methods and Analysis
This is a prospective, observational study to determine whether previously identified makers of susceptibility to nosocomial infection can be validated in a multi-centre population, as well as testing several novel markers which may improve the risk of nosocomial infection prediction. Blood samples from critically ill patients (those admitted to ICU for at least 48 hours and require mechanical ventilation alone or support of two or more organ systems) are taken and undergo whole blood staining for a range of immune cell surface markers. These samples undergo analysis on a standardised flow cytometry platform. Patients are followed up to determine whether they develop nosocomial infection. Infections need to meet strict pre-specified criteria based on international guidelines, where these criteria are not met an adjudication panel of experienced intensivists are asked to rule on presence of infection. Secondary outcomes will be death from severe infection (sepsis) and change in organ failure.

Ethics and Dissemination
Ethical approval including the involvement of adults lacking capacity has been obtained from respective English and Scottish Ethics Committees. Results will be disseminated through presentations at scientific meetings and publications in peer reviewed journals.

Registration

The study is registered on clincialtrials.gov with number NCT02186522

Strengths and Limitations

Strengths

- Multi-site study, so increasing the generalisability of results obtained.
- Multi-site nature has produced a program of flow cytometry standardisation which we believe to be both robust and reproducible and so set the scene for potential clinical use of these assays should they prove to be of value.
- We have taken steps to try to minimise variability in the diagnosis of infection, through the use of rigid criteria and consensus review of cases which do not meet these criteria.

Limitations

The weaknesses of this study are that it is observational, and thus will not be able to inform clinicians of what actions they should take in response to these results should clinically useable tests be developed.

Introduction

Critical illness increases the risk of nosocomial infection, with between 20 and 40% of patients admitted to ICU acquiring infections during their critical care stay (1,2), a rate that approaches that seen in hematopoietic stem cell transplantation (3). Provision of organ support requires the disruption of epithelial and mucosal barrier innate immune system protection through the placement of devices such as endo-tracheal tubes, urinary catheters and central venous catheters. These infections are often bacterial and are associated with increased antibiotic use. (1, 2). In addition to bacterial infections, critically ill patients are at risk of reactivation of latent viral infections (4). Therefore, it is thought that the combination of immune vulnerability and microbial colonization are responsible for the high rates of nosocomial infection seen in critically ill patients (1).

Critical illness resulting from trauma, sepsis and post surgical complications all have commonality in the innate and adaptive immune responses. (5,6) Many diseases that can precipitate the need for exogenous organ support and admission to intensive care are characterized by a profound systemic inflammatory response (7), with associated immune cell activation (8) and immune system-mediated organ damage (9). However it is now increasingly apparent that this over-exuberant inflammation is accompanied by an equally vigorous counter-regulatory anti-inflammatory response (5,10). The apparently maladaptive, complex immune dysfunction in critically ill patients manifests across a range of cellular actions and functions, involving both the innate and adaptive arms of the immune system (5,10-15). Defects have been noted in neutrophils (11), monocytes (12,16,17), T lymphocytes (10, 13-15) and splenic dendritic cells (10). The recent identification of elevated proportions of regulatory helper-T cells (Tregs) in sepsis (14,15) is in keeping with the supposition that much of the immunosuppression arises from the over-activation of counter-regulatory mechanisms. In human and experimental sepsis, Tregs mediate lymphocyte anergy and are associated with worse outcomes (15).

Rationale for the study

Although it seems plausible that the immune defects found in critical illness are associated with an increased risk of acquiring nosocomial infections, the concept that immune cell abnormalities always precede nosocomial infections, necessary for causality, is inconsistently reported in the literature (14,16). Following our previous study (11), which demonstrated the benefits of combining measures of immune dysfunction on predicting nosocomial infection, there is a need to validate the markers in a new cohort. Undertaking this as a multi-centre study will help develop standardised flow cytometric measures and improve external validity of the markers that predict risk of nosocomial infection. If a selected set of immune abnormalities that could be modified with a single intervention are present together, then development of a test could stratify patients for risk of nosocomial infections for targeted interventions (17). Finally, identifying

multiple cell defects / markers will help design future trials of therapies and prophylactic measures to prevent nosocomial infections.

Hypotheses

1 Markers of neutrophil, monocyte and T-cell hypoactivity will predict those critically ill patients who are at risk of nosocomial infection.
2 These markers will have added predictive value when combined
3 Additional predictive ability will be achieved through examination of monocyte and Treg subsets and other cell surface markers of immune cell function.

Primary aims

1 To validate the ability of neutrophil CD88, monocyte HLA-DR and percentage of Tregs to predict those patients at risk of nosocomial infection
2 To determine the optimal way of combining these measures to risk stratify patients
3 To develop a clinically useable test

Secondary aims

1 To determine the relationship between the measures outlined above and risk of death from sepsis
2 To determine whether more detailed phenotyping of monocyte and Treg subsets provides additional value in predicting risk of nosocomial infection
3 To explore whether other cell surface measures of immune cell function and phenotype may predict nosocomial infection.

Methods and analysis

This protocol outlines a multi-centre, prospective observational study in which critically ill patients will be recruited and assessments made of immune cell surface phenotypic markers at multiple time points. Patients will be followed to determine outcomes of interest, the primary outcome being development of nosocomial infection.

Study population

The population will be drawn from 4 UK adult intensive care units, consisting of:
1 Royal Infirmary, Edinburgh (liver/general unit)
2 Western Infirmary, Edinburgh (neurosciences/general unit)
3 St Thomas's Hospital, London (cardiac/general unit)
4 Sunderland Royal Hospital (general unit)

Consecutive patients will be eligible if they are age >16 (Scotland) or >18 (England), receiving support of level 3 care (i.e. requiring invasive support of

respiratory system alone, or two or more other organ systems (haemofiltration, inotropes/vasopressors) and predicted to remain in ICU for at least 48 hours.

Exclusion criteria are not being expected to survive for a further 24 hours, known or suspected ICU-acquired infection at time of screening (non-ICU acquired nosocomial infection – i.e. non-ICU healthcare associated infection is not an exclusion), known inborn errors of immune function, immunosuppression (corticosteroids up to 400mg hydrocortisone equivalent daily dose permitted), HIV infection, hepatitis B and C infection, receiving Extra-Corporeal Membrane Oxygenation (ECMO), pregnancy and previously enrollment in the study.

Co-enrolment is permitted where the overall phlebotomy burden on patients is acceptable (<50ml at any one time point and total phlebotomy load of <150ml), where the co-enrolled study is deemed unlikely to affect the primary endpoint of the INFECT study and where a formal co-enrolment agreement is in place.

Sampling schedule

Blood samples for flow cytometric analysis of surface receptor expression will be taken on study enrolment, and then on day 2 after study enrolment, and at 48 hour intervals until day 12, a maximum of 7 samples per patient. Patients discharged from ICU within the 16 day study window will have a maximum of 2 further samples taken at 3-4 day intervals up to day 12 of study to minimize burden of venepuncture. Where possible these will be collected at the time of routine venepuncture for clinical sampling. Patients will be followed for the development of infection for 16 days (by this time the great majority of infections had been acquired in the original study and few patients were left in the ICU alive and without infection). All survivors who remain in hospital will be followed to this point including those who have left ICU.

Clinical and demographic data will be collected including age, sex, functional co-morbidity index, smoking status, physiological data (APACHE II score on admission, SOFA score at baseline, full blood count and differential white cell count) and clinical data (admission diagnosis, admission source, antibiotics, invasive devices present and duration). Similar clinical data will be collected at each sample time point. Hospital outcome (i.e. discharged alive, died or transferred to another hospital) will be also be collected.

Definition of infection

Infections will need to meet strict, pre-defined criteria (see appendix A below) and will be assessed by research staff blinded to the immune phenotyping data. Suspected infections which do not meet the criteria for confirmed infection will be reviewed 'off-line' by a panel of clinicians blinded to the immune phenotyping data using information from a prospectively collected pro-forma. The outcomes from this will be "highly likely" infection and "unlikely infection/colonisation" (colonisation being where microbes are grown in the absence of evidence for infection). The day infection is acquired will be defined as the day the sample which shows positive for microbiology was taken. In the case of sterile cultures where the clinician strongly suspects infection the day of infection will be

defined as the day strong clinical suspicion was raised. Therefore outcomes will be a) "confirmed", b) "probable infection" and c) "unlikely infection/colonisation".

Patients who are transferred to a non study hospital will have data included up until day of discharge and will be followed up via telephone contact from the recording unit to ascertain whether there were any confirmed or suspected infections in the days following transfer (up to 16 days post-study entry). Patients transferred from one participating site to another will remain in the study and have data and samples collected as per study protocol.

Flow cytometric standardisation and sample staining

All sites have standardised on the same platform, the FACS Canto II (Becton Dickinson Biosciences, San Jose, CA, USA - from here on BDB), for flow cytometric analysis of samples. Machines will be standardised by monthly matching of target values using a common batch of Cytometry Setup and Tracking (CS&T) beads (BDB), and daily internal quality control using CS&T beads.

Leukocyte cell surface staining will be conducted using antibodies supplied by BDB. All sites use antibodies from the same batch. Staining, data capture and storage will be conducted in accordance with a single study standard operating procedure.

Flow cytometry data will be held centrally. Final analysis will be conducted using a single analysis platform, VenturiOne (Applied Cytometry, Sheffield, UK).

Cellular immunophenotyping.

The primary measures are to validate our previous findings (11), namely: neutrophil CD88, monocyte HLA-DR, and percentage CD4 cells expressing the CD25+/CD127lo regulatory phenotype, using the cut-offs defined by our previous study (11).

Additional phenotypic measurements include: neutrophil and monocyte activation markers (including CD11b, CD66b, CD312), sub-types of regulatory T-cells and Treg activation, frequency of monocyte sub-sets and subset HLA-DR expression, frequency of dendritic cell sub-types, expression of monocyte and lymphocyte PD1 and PDL1, frequency of granulocytic and Monocytic myeloid derived suppressor cells, and frequency of B-cell subtypes.

Sample size

In a cohort studied previously (11), 34% of patients were confirmed as having secondary sepsis. We therefore expect the panel of markers to perform well when predicting 25%-45% of patients to have secondary sepsis. Across a broad range of possible positive predictive value (PPV) performance (50%-90%), the 95% confidence interval width for the PPV would range from ±5.5% to ±15.9%,

indicating moderately precise estimation of the PPV. Using a similar rationale, we would expect the 95% confidence interval width for the negative predictive value (NPV) to range from ±4.3% to ±10.8%, indicating precise estimation of the NPV. A conservative estimate of a 50% PPV with a 35% rate of secondary sepsis would yield a 95% confidence interval of 39-61% in a cohort of 200 patients. We also intend to recruit 20 patients initially to confirm the reformulated flow cytometric tests match the performance of those from the derivation cohort (11) and thus we propose recruiting to a 220 patient cohort

Informed consent

Consent and assent procedures will be conducted under the relevant legislation; in England (Mental Capacity Act, 2005) or Scotland (Adults with Incapacity (Scotland) Act, 2000) for consent/assent of adults without capacity. In England assent is obtained, where possible, following discussion with the patient's next of kin (personal consultee). Where a personal consultee is unavailable assent is provided by a professional consultee, being a senior medical professional who is not in the research team. In circumstances where next of kin are unable to attend the ICU promptly, deferred consent procedures are used.

In Scotland the patient's relative or welfare attorney provides consent. If the patient's relative or welfare attorney is unable to attend the ICU, consent may be provided in a telephone conversation providing a second member of staff witnesses the discussion.

Patients that recover capacity will be approached to provide retrospective consent.

Safety of participants

The only safety concern is that of potential additional venepuncture in patients, the risks of this are of minor harm (bruising). Post-ICU sampling (where indwelling lines are not present for blood sampling) is limited to a maximum of two samples.

Data analysis plan

A detailed statistical analysis plan will be finalised prior to the locking of the study database.

Studies will be conducted to ensure the flow cytometric readings of each marker are reproducible to demonstrate they can be a useful test. Inter- and intra-observer reliability studies will be conducted with three expert readers of the data. 50 data files will be read to determine inter-observer agreement, with 13 files for intra-observer agreement. After a preliminary reliability study, an optimisation stage will occur with expert meetings to further improve flow cytometric gating strategies in problematic markers to ensure we do not wrongly reject markers. A second reliability study will then be conducted using the same sample size to select markers taken forward to future stages of analysis.

The main analysis of the primary outcome will be an analysis of the PPV and NPV of immune dysfunctions in predicting nosocomial infection, as well as the predictive ability of combinations of immune dysfunction. The primary analysis will include both 'confirmed' and 'probable' infections as 'infections', with analysis by 'confirmed' infections only conducted as a sensitivity analysis. Sensitivity and specificity will also be determined. 95% confidence intervals will be calculated for all measures of predictive accuracy.

As with the derivation cohort (6), 'immune function/dysfunction' will be defined by the time point before first nosocomial infection occurs, censored for two days prior to infection.

As a secondary analysis a Cox model of time to acquisition of infection will be fitted, with the classification 'immune dysfunction' or 'no dysfunction' as one independent variable. The other independent variables will be potential clinical confounders identified in previous epidemiological work looking at risk factors for nosocomial infection (1,2) and will demonstrate whether our novel tests add predictive value over routinely available clinical and demographic data.

The association of immune dysfunctions with the secondary outcome measures ICU outcome (lived/died) and death from sepsis (yes/no) will be assessed using the same methods as the main analysis of the primary outcome.

Determination of the clinical utility of the test will come from a two-stage assessment. First the reliability of the flow cytometric markers will be assessed, markers for clinical use must have excellent inter and intra-rater reliability. Second the markers must be clinically valuable, adding predictive ability beyond that which can be gained from clinical assessment and standard laboratory parameters. Both these assessments are inherent in the data analysis program outlined above, and will be reviewed both internally by the study consortium and also by a group of key clinical stakeholders who are independent of the study consortium. Alongside this a mocked-up clinical workflow will be developed based on the laboratory procedures developed to run this study, using the experience of Becton Dickinson's established clinical assay systems.

Ethics and dissemination

The study has been approved by the Scotland A research ethics committee (13/SS/0022) for Scottish sites and Warwick and Coventry research ethics committee (13/WM/0207) for English sites. Local research governance approval has been granted by the 3 NHS organisations covering the 4 sites where the study is being conducted.

Study management

The study is managed by the Edinburgh Clinical Trials Unit (ECTU) and the Edinburgh Critical Care Research Group (ECCRG) at the University of Edinburgh, Edinburgh, Scotland.

Sponsorship

The study is co-sponsored by University of Edinburgh and NHS Lothian.

Duration of study

The study was planned to run for 3 years, starting October 2012, with initial assay development leading to patient recruitment starting July 2014. Following successful requests for grant extension due to delays in setting up recruiting sites the expected end date for recruitment is January 31st 2016. Flow cytometric data will require post-acquisition processing prior to analysis, this is expected to complete by 1st April 2016. We expect to have completed our primary data analysis with a view to dissemination of results by August 2016, and at this point we will consider the study complete.

Results from the study will be reported according to the STARD[18] guidelines for reporting diagnostic accuracy studies and disseminated via presentations at scientific meetings and publications in peer reviewed journals

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Registration

The study is registered on clincialtrials.gov with number NCT02186522

Author's contributions

Study conception (TW, NW, AB, ACM), obtaining funding (TW, NW, JK, AB, JS, ACM), development of flow cytometry techniques (JR, AW, AR, SL, TM, GH, ID), development of statistical analysis plan (TW, DD, CJW, ACM), protocol development (TW, DD, JA, AR, NW, JK, MSH, AB, JS, ID, ACM) manuscript drafting (DD, ACM). All authors have reviewed and approve the final version.

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Appendix A- definitions of infection

Any new infection occurring after 48 hours of ICU admission will be deemed 'ICU-acquired'. For consistency infections arising within 48 hours of ICU discharge will also deemed 'ICU-acquired'.

Infections will be defined prior to start of the study as follows, based on the Hospitals in Europe Link for Infection Control through Surveillance (HELICS) criteria.

a) Ventilator-associated pneumonia: Requires radiographic, clinical and microbiological criteria to be met:

i. Radiological criteria.
CXR or CT scan showing new infiltrates, or worsening infiltrates without evidence of pulmonary oedema, and either pyrexia of >38^{o}C or white cells >12000/mm^3 or <4000/ mm^3.

These must be combined with one or more clinical criteria.

ii. Clinical criteria.
Worsening oxygenation – any increase in FiO_2 to maintain PaO_2 target, or an increase in PEEP, frequency or tidal volume, proning or paralysis to facilitate ventilation.
OR
Relevant clinical chest findings – auscultatory finding of crepitations, crackles or decreased air entry.
OR
Increased/changed sputum – any increase in volume, presence of muco-purulent or muco-purulent-bloody sputum.

iii. Microbiological criteria.
The above radiological and clinical criteria must be combined with positive quantitative BAL culture of >10^{4}CFU/ml (of >10^{3}CFU/ml on protected specimen brush (PSB) sampling) or positive pleural fluid or pulmonary/pleural abscess culture.
Where the diagnosis of VAP has been suggested by mini-BAL, endotracheal aspirate or where growth is below the 10^4 CFU/ml threshold or without any positive microbiology, adjudication is required.

Hospital-acquired pneumonia (HAP), i.e. nosocomial pneumonia in non-mechanically ventilated patients (or patient in ICU for >48 hours but ventilated for <48 hours), requires the same fulfilment of criteria as VAP except that sputum cultures with heavy growth of a single organism constitute a confirmed infection.

b) Catheter-associated infections
Positive culture (where semi-quantitative cultures available >15CFU) from an indwelling vascular line combined with either
Local inflammation and pus (catheter-related infection (CRI)) or

Improvement of inflammatory markers within 48 hours of removal (CRI) or Culture of the same organism from a peripheral blood culture (catheter-related blood stream infection (CRBSI).

c) Blood stream infection
One positive culture of a typical pathogen, coupled with evidence of systemic inflammation (WCC >12,000/mm^3 or <4000/mm^3, temperature ≥ 38^{o}C).

d) Urinary tract infection
Growth of 2 or fewer organisms at ≥ 10^5 CFU/ml combined with evidence of systemic inflammation (WCC >12,000/mm^3 or <4000/mm^3, temp >38^{o}C or shock without another identifiable cause).

e) Soft-tissue or surgical site infection
Evidence of pus/inflammation at site of presumed infection combined with a positive culture.

f) *Infective diarrhoea*
Evidence of diarrhoea (3 or more loose stools in 24 hour period or use of a feacal collector) combined with culture positive for diarrhoeal organism or detection of enteropathogenic toxin.

g) *Intra-abdominal infection*
Evidence of intra-abdominal collection identified on surgical or radiological investigation, combined with positive culture from surgical specimen, needle aspirate or drain.

h) *Spontaneous bacterial peritonitis (SBP)*
Evidence of infected ascites (fluid with >250 neutrophils/mm^3, or abdominal pain and blood WCC >12,000/mm^3 or <4000/mm^3, temperature ≥ 38^{o}C in the presence of ascites without other source of infection) combined with positive ascitic fluid culture. Negative ascitic fluid culture in the presence of clinical criteria for SBP would be 'probable' infection.

i) *Sinusitis*
Evidence of facial sinus fluid collection on radiological (plain film, CT or ultrasound) or direct clinical observation of purulent discharge from a sinus combined with positive culture of fluid.

Infecting organisms may be bacterial, fungal or viral. Viral infections must be accompanied by clinical, radiological or histological evidence of tissue inflammation (e.g. herpes simplex stomatitis, Varicella pneumonitis, CMV colitis). Viral positivity without evidence of tissue inflammation would be classified as reactivation.

A 'confirmed' infection is one which meets the above criteria

Where an infection is suspected by the clinical team but does not meet the criteria above the case will require a proforma completed and details sent to

members of an expert consensus panel who are blinded to the immune cell data. The proforma will be reviewed by two members of the panel who will independently adjudicate the infection as either 'probable' or 'unlikely', in the event of the judgements being different a third panel member will be asked with the majority decision being recorded.

A 'probable' infection is where the panel clinician thinks there is, on the balance of probabilities, an infection present and would consider antibiotic treatment and/or source control if the patient's clinical condition merited it. This category may include positive microbial cultures. An example would be culture of a classically non-pathological organism (e.g. single cultures of coagulase negative cocci or diphtheroids) associated with clinical evidence of infection/systemic inflammation. Classically non-pathological organisms can be classified as 'confirmed' infections where there is strong evidence

An 'unlikely' infection is where the panel clinician thinks there is a low probability of infection and would not consider antibiotic treatment and/or source control. Although positive microbial cultures could be included in this, this would be culture of a classically non-pathological organism (e.g. single cultures of coagulase negative cocci or diphtheroids) without evidence of systemic inflammation/infection or mixed growth of commensal organisms.

Systemic evidence of infection would require the presence of SIRS – specifically 2 or more of the following heart rate>90 beats per minute, WCC>12/mm^3 or <4/mm^3 or >10% band types, respiratory rate >20 breaths per minute or mechanical ventilation, and temperature of >38°C or <36°C. Additional evidence to consider would include reports of large numbers of neutrophils on sample microscopy, and clinical examination findings of pus or inflamed tissue.

'Unlikely' infection combined with a positive microbial culture would constitute colonisation.

Section & Topic	No	Item	Reported on page #
TITLE OR ABSTRACT			
	1	Identification as a study of diagnostic accuracy using at least one measure of accuracy (such as sensitivity, specificity, predictive values, or AUC)	1
ABSTRACT			
	2	Structured summary of study design, methods, results, and conclusions (for specific guidance, see STARD for Abstracts)	2
INTRODUCTION			
	3	Scientific and clinical background, including the intended use and clinical role of the index test	4
	4	Study objectives and hypotheses	5
METHODS			
Study design	**5**	Whether data collection was planned before the index test and reference standard were performed (prospective study) or after (retrospective study)	5
Participants	**6**	Eligibility criteria	5
	7	On what basis potentially eligible participants were identified (such as symptoms, results from previous tests, inclusion in registry)	5
	8	Where and when potentially eligible participants were identified (setting, location and dates)	5
	9	Whether participants formed a consecutive, random or convenience series	5
Test methods	**10a**	Index test, in sufficient detail to allow replication	7
	10b	Reference standard, in sufficient detail to allow replication	Na
	11	Rationale for choosing the reference standard (if alternatives exist)	Na
	12a	Definition of and rationale for test positivity cut-offs or result categories of the index test, distinguishing pre-specified from exploratory	7
	12b	Definition of and rationale for test positivity cut-offs or result categories of the reference standard, distinguishing pre-specified from exploratory	7
	13a	Whether clinical information and reference standard results were available to the performers/readers of the index test	6
	13b	Whether clinical information and index test results were available to the assessors of the reference standard	6
Analysis	**14**	Methods for estimating or comparing measures of diagnostic accuracy	8
	15	How indeterminate index test or reference standard results were handled	Na
	16	How missing data on the index test and reference standard were handled	
	17	Any analyses of variability in diagnostic accuracy, distinguishing pre-specified from exploratory	8
	18	Intended sample size and how it was determined	8
RESULTS			
Participants	**19**	Flow of participants, using a diagram	Na
	20	Baseline demographic and clinical characteristics of participants	Na
	21a	Distribution of severity of disease in those with the target condition	Na
	21b	Distribution of alternative diagnoses in those without the target condition	Na
	22	Time interval and any clinical interventions between index test and reference standard	Na
Test results	**23**	Cross tabulation of the index test results (or their distribution) by the results of the reference standard	Na
	24	Estimates of diagnostic accuracy and their precision (such as 95% confidence intervals)	Na
	25	Any adverse events from performing the index test or the reference standard	Na
DISCUSSION			
	26	Study limitations, including sources of potential bias, statistical uncertainty, and generalisability	Na
	27	Implications for practice, including the intended use and clinical role of the index test	Na
OTHER INFORMATION			
	28	Registration number and name of registry	9
	29	Where the full study protocol can be accessed	9
	30	Sources of funding and other support; role of funders	9

STARD 2015

AIM

STARD stands for "Standards for Reporting Diagnostic accuracy studies". This list of items was developed to contribute to the completeness and transparency of reporting of diagnostic accuracy studies. Authors can use the list to write informative study reports. Editors and peer-reviewers can use it to evaluate whether the information has been included in manuscripts submitted for publication.

EXPLANATION

A **diagnostic accuracy study** evaluates the ability of one or more medical tests to correctly classify study participants as having a **target condition.** This can be a disease, a disease stage, response or benefit from therapy, or an event or condition in the future. A medical test can be an imaging procedure, a laboratory test, elements from history and physical examination, a combination of these, or any other method for collecting information about the current health status of a patient.

The test whose accuracy is evaluated is called **index test.** A study can evaluate the accuracy of one or more index tests. Evaluating the ability of a medical test to correctly classify patients is typically done by comparing the distribution of the index test results with those of the **reference standard**. The reference standard is the best available method for establishing the presence or absence of the target condition. An accuracy study can rely on one or more reference standards.

If test results are categorized as either positive or negative, the cross tabulation of the index test results against those of the reference standard can be used to estimate the **sensitivity** of the index test (the proportion of participants *with* the target condition who have a positive index test), and its **specificity** (the proportion *without* the target condition who have a negative index test). From this cross tabulation (sometimes referred to as the contingency or "2x2" table), several other accuracy statistics can be estimated, such as the positive and negative **predictive values** of the test. Confidence intervals around estimates of accuracy can then be calculated to quantify the statistical **precision** of the measurements.

If the index test results can take more than two values, categorization of test results as positive or negative requires a **test positivity cut-off**. When multiple such cut-offs can be defined, authors can report a receiver operating characteristic (ROC) curve which graphically represents the combination of sensitivity and specificity for each possible test positivity cut-off. The **area under the ROC curve** informs in a single numerical value about the overall diagnostic accuracy of the index test.

The **intended use** of a medical test can be diagnosis, screening, staging, monitoring, surveillance, prediction or prognosis. The **clinical role** of a test explains its position relative to existing tests in the clinical pathway. A replacement test, for example, replaces an existing test. A triage test is used before an existing test; an add-on test is used after an existing test.

Besides diagnostic accuracy, several other outcomes and statistics may be relevant in the evaluation of medical tests. Medical tests can also be used to classify patients for purposes other than diagnosis, such as staging or prognosis. The STARD list was not explicitly developed for these other outcomes, statistics, and study types, although most STARD items would still apply.

DEVELOPMENT

This STARD list was released in 2015. The 30 items were identified by an international expert group of methodologists, researchers, and editors. The guiding principle in the development of STARD was to select items that, when reported, would help readers to judge the potential for bias in the study, to appraise the applicability of the study findings and the validity of conclusions and recommendations. The list represents an update of the first version, which was published in 2003.

More information can be found on http://www.equator-network.org/reporting-guidelines/stard.