Pro/Con debate on Clinical Metagenomics

Wake me up when it’s over?

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Arguments against cMGx

- Linguistics
- Sample prep and workflow
- Bioinformatics and data management
- Communications
- Clinical impact
- Costs and economics
- Human resource
- Quality control and quality assurance
- Regulation and accreditation
Linguistics

• **Metagenomic** analysis or **metagenomics** analysis?
• **Metagenomic** tools or **metagenomics** tools?
• **Metagenomic** sequencing or **metagenomics** sequencing?
• Metagenomics – an attributive noun or adjective?
• What linguistic experts say?
• Key question:

  *Can a new technology be ready for clinical use when it is not even sorted out grammatically?!*
Clinical metagenomics of bone and joint infections: a proof of concept study

Etienne Ruppert, Vladimir Lazarevic, Myriam Girard, William Mouton, Tristan Ferry, Frédéric Laurent & Jacques Schrenzel

Discussion

The main result of this study is that we showed that metagenomic sequencing could be a potential tool in the diagnostic of BJI. Indeed for monomicrobial infections, the pathogen was identified in 100% (8/8) samples and the antibiotic susceptibility prediction was successful in 94.1% (111/128) cases. In case of polymicrobial samples, the high abundance of several bacteria (mostly anaerobes) did occasionally prevent from the correct identification of the pathogens and their antibiotic susceptibility profiles. Accordingly, our findings support that currently, metagenomic sequencing of BJI samples could not replace conventional methods based on culture due to the limitations encountered when several bacterial species are present in the study, but rather be performed in support.

Interestingly, metagenomic sequencing yielded in some ways more information than culture. First, metagenomic sequencing identified many more bacterial species than culture. Besides likely contaminants, some bacteria that were not detected by culture were probably true positive and may not have been targeted by the selected antibiotic regimen, as we observed in 10% cases. Second, we could identify multiple clonal populations within some species, which could differ in their susceptibility to antibiotics as we observed for fluorquinolones in M. morganii. Sequencing multiple clones obtained from the culture of BJI samples would validate this finding and shall be considered for further studies. In all, using metagenomic data could help to tailor the antibiotic regimen for the treatment of BJI, and the added-value of clinical metagenomics in BJI should now be assessed.

Critical steps in clinical shotgun metagenomics for the concomitant detection and typing of microbial pathogens

Natacha Couto, Leonard Schuelle, Erwin C. Raangs, Miguel P. Machado, Catarina I. Mendes, Tiago F. Jesus, Monika Chlebovicz, Sigrid Rosema, Mário Ramírez, João A. Carrió, Ingo B. Autenrieth, Alex W. Friedrich, Silke Peter & John W. Rossen

Methods

Sample collection. Nine body fluid samples and one tissue sample entering the Medical Microbiology laboratory were selected for metagenomics sequencing. These included one sample from peritoneal fluid, five from pus (3 abscesses and 2 empyema), two from synovial fluid of knees with prosthesis, one from sputum and one from a bone biopsy (Table 1). All samples were stored at 4°C for a variable period (2–10 days). The samples used for the present analyses were collected during routine diagnostics and infection prevention and control investigations. All procedures were carried out according to guidelines and regulations of UMCG concerning the use of patient materials for the validation of clinical methods, which are in compliance with the guidelines of the Federation of Dutch Medical Scientific Societies (FDMSS). Every patient entering the UMCG is informed that samples taken may be used for research and publication purposes, unless they indicate that they do not agree to it. This procedure has been approved by the Medical Ethical Committee of the UMCG. Informed consent was obtained from all individuals or their guardians prior to study participation. All samples were used after performing and completing a conventional microbiological diagnosis and were coded to protect patients' confidentiality. All experiments were performed in accordance with the guidelines of the Declaration of Helsinki and the institutional regulations.
Sample prep and workflow

• Differing performance between protocols of extraction, library prepping and sequencing

• Background DNA impacts sensitivity, requires greater depth – enrichment? depletion? brute force approach?

• Challenge of specimens from non-sterile sites – needle in a haystack!

• Contamination, low biomass specimens, kitome issues

• And what about quantitation?
Sample prep and workflow

• Study explored impact of different crossed over workflows on mNGS
• Not all methods work equally well for different pathogens
• Bead beating!
• WGA
• Negative extraction controls
Sample prep and workflow

### Table 2. Some of the Variables That Influence Analytic Sensitivity

<table>
<thead>
<tr>
<th>Dependent on...</th>
<th>Test Design</th>
<th>Pathogen</th>
<th>Specimen Type</th>
<th>May Vary by Specimen</th>
</tr>
</thead>
<tbody>
<tr>
<td>Effectiveness of nucleic acid extraction</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Pathogen genome size</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Efficiency of library preparation</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Number of sequence reads</td>
<td>X</td>
<td></td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>Availability of reference sequences</td>
<td></td>
<td>X</td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>Sequence uniqueness</td>
<td></td>
<td></td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Accuracy of classification algorithms</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Required confidence for identification</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Specimen composition</td>
<td></td>
<td></td>
<td>X</td>
<td>X</td>
</tr>
</tbody>
</table>

Abbreviation: X, variable influences analytic sensitivity.

A

B

*Pathogen*

*Human cells*

*IC*
Bioinformatics and data management

• Too many bioinformatics tools, black screens, black boxes
• Software (freeware) developers are commonly not clinically oriented
• No standardisation of analysis
• Computational challenges remain (e.g. resistome, MAGs, non-sterile sites)
• Analysis outputs require significant post-processing, interpretation (genotype to phenotype correlation, polymicrobial, weird taxa, few reads)
• Databases incomplete, not fit-for-purpose
• Data storage and data sharing challenges, computing power
• Data safety and security, human genome component
Bioinformatics and data management

### Table 1. A List of Benchmarked Classifiers and Their Various Characteristics

<table>
<thead>
<tr>
<th>Type</th>
<th>Classifier</th>
<th>Custom Databases</th>
<th>Generates Abundance Profile</th>
<th>Memory Required</th>
<th>Time Required</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNA</td>
<td>Bracken</td>
<td>yes</td>
<td>yes</td>
<td>&lt;1 Gb</td>
<td>&lt;1 min</td>
<td>Lu et al., 2017</td>
</tr>
<tr>
<td></td>
<td>Centrifuge</td>
<td>yes</td>
<td>yes</td>
<td>20 Gb</td>
<td>7 min</td>
<td>Kim et al., 2016</td>
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<tr>
<td></td>
<td>CLARK</td>
<td>yes</td>
<td>yes</td>
<td>80 Gb</td>
<td>2 min</td>
<td>Ounit et al., 2015</td>
</tr>
<tr>
<td></td>
<td>CLARK-S</td>
<td>yes</td>
<td>yes</td>
<td>170 Gb</td>
<td>40 min</td>
<td>Ounit and Lonardi, 2016</td>
</tr>
<tr>
<td></td>
<td>Kraken</td>
<td>yes</td>
<td>yes</td>
<td>190 Gb</td>
<td>1 min</td>
<td>Wood and Salzberg, 2014</td>
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<td></td>
<td>Kraken2</td>
<td>yes</td>
<td>yes</td>
<td>36 Gb</td>
<td>1 min</td>
<td>Wood and Salzberg, 2014</td>
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<tr>
<td></td>
<td>KrakenUniq</td>
<td>yes</td>
<td>yes</td>
<td>200 Gb</td>
<td>1 min</td>
<td>Breitwieser et al., 2018</td>
</tr>
<tr>
<td></td>
<td>k-SLAM</td>
<td>yes</td>
<td>yes</td>
<td>130 Gb</td>
<td>2 h</td>
<td>Ainsworth et al., 2017</td>
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<tr>
<td></td>
<td>MegaBLAST</td>
<td>yes</td>
<td>no</td>
<td>61 Gb</td>
<td>4 h</td>
<td>Morgulis et al., 2008</td>
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<tr>
<td></td>
<td>metaOrthologs</td>
<td>no</td>
<td>no</td>
<td>30 Gb</td>
<td>1 min</td>
<td>Liu et al., 2018</td>
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<tr>
<td></td>
<td>PathSeq</td>
<td>yes*</td>
<td>no</td>
<td>140 Gb</td>
<td>5 min</td>
<td>Walker et al., 2018</td>
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<tr>
<td></td>
<td>prophylo</td>
<td>yes</td>
<td>no</td>
<td>40 Gb</td>
<td>40 min</td>
<td>Bfini et al., 2017</td>
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<tr>
<td></td>
<td>taxTrees</td>
<td>yes</td>
<td>yes</td>
<td>65 Gb</td>
<td>25 min</td>
<td>Corvelo et al., 2018</td>
</tr>
<tr>
<td>Protein</td>
<td>DIAMOND</td>
<td>yes</td>
<td>no</td>
<td>110 Gb (varies)</td>
<td>10 min</td>
<td>Buchholtz et al., 2015</td>
</tr>
<tr>
<td></td>
<td>Kaju</td>
<td>yes</td>
<td>yes</td>
<td>25 Gb</td>
<td>1 min</td>
<td>Mertel et al., 2016</td>
</tr>
<tr>
<td></td>
<td>MMseqs2</td>
<td>yes</td>
<td>no</td>
<td>85 Gb (varies)</td>
<td>9 h</td>
<td>Steinegger and Söding, 2017</td>
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<tr>
<td></td>
<td>Markers</td>
<td>yes</td>
<td>yes</td>
<td>2 Gb</td>
<td>1 min</td>
<td>Truong et al., 2015</td>
</tr>
<tr>
<td></td>
<td>mOTU2s</td>
<td>no</td>
<td>yes</td>
<td>2 Gb</td>
<td>1 min</td>
<td>Milanese et al., 2019</td>
</tr>
</tbody>
</table>

Custom databases refers to the ability for the end user to create a custom database. The time and memory requirements are for a 5.7 million-read dataset with the database and input already cached in memory. Some methods (marked as “varies”) have the ability to flexibly decrease their memory usage (at the cost of a massive increase in run time).

*The latest version of PathSeq now allows the user to create and specify a custom database, but this option was not available when benchmarking studies were performed; thus, it was excluded from those analyses.
Communications

• Hard to sell cMGx to clinicians, explain TAT and expected results
• Difficult to communicate to other stakeholders (e.g. management)
• Challenges of explaining test performance and limitations (assuming that we know them), no magic bullet
• Challenges of disseminating complex results
Clinical impact

• Anecdotal cases don’t make EBM!
• Very few comparative head-to-head studies
• Very few real-life prospective studies, no RCTs
• Choice of control group or comparator
• Extremely limited data on outcome improvement (as opposing to accurate diagnosis)
• WH questions of testing not agreed
• From NNT to NNS
• Risk of increasing TATs due to work protocols, complicated analyses, batching of samples – deleterious to patients
Clinical impact

• The NEJM paper: the most extensive cMGx study to date
• Convenience sample, no sample size calculation
• Compared to standard and orthogonal lab confirmation
• Weekly testing regimen + board discussion
• Focus on CNS - meningitis / encephalitis / myelitis et. al
• Samples collected after median of 3d (0-219)
• No data presented on TAT, repeats, direct costs, overall costs and C/E

• Conclusion: Routine microbiologic testing is often insufficient to detect all neuroinvasive pathogens. In this study, metagenomic NGS of CSF obtained from patients with meningitis or encephalitis improved diagnosis of neurologic infections and provided actionable information in some cases

Wilson et al., NEJM 2019
Clinical impact

Wilson et al., NEJM 2019
Clinical impact

E Clinical Effect (13 cases diagnosed by metagenomic NGS only)

- **N. farrincia** — long-term treatment with oral moxifloxacin and minocycline
- **Candida tropicalis** — treatment with high-dose fluconazole and liposomal amphotericin B (started empirically for elevated 1,3-β-D-glucan level)
- **HEV** — successful treatment with IV ribavirin after patient was readmitted with liver failure and consideration of liver transplantation
- **E. aerogenes** — narrowing of antibiotic therapy to IV cefepime and oral trimethoprim–sulfamethoxazole
- **Enterococcus faecalis** — narrowing of antibiotic therapy to IV vancomycin; discontinuation of meropenem
- **S. mitis** — narrowing of antibiotic therapy to IV cefepime; continuation of antibiotics for 4 wk to treat CNS infection
- **S. agalactiae** — treatment with an additional 4 wk of therapy with IV ceftriaxone and vancomycin

- **7 (54%)** Enabled appropriate and targeted treatment
- **1 (8%)** Helped to rule out coinfections; enabled patient to proceed with chemotherapy (EBV-associated lymphoma)
- **1 (8%)** Supported clinical decisions to narrow coverage (neisseria)
- **2 (15%)** Had no effect, because patient already discharged from hospital (enterovirus)
- **1 (8%)** Had no effect, because clinical significance unclear (MW polymavirus)
- **1 (8%)** Provided reassurance to patient or surrogate (SLEV)
Clinical impact

- Cohort of ABM (N=135)
- Sp diagnosed in 43
- Culture 26, BINAX 35, mNGS 32
- mNGS only – 6 cases
- Overall agreement – 26+92 (87%)
- Some confirmation with qPCR attempted but no consistent comparison (e.g. FilmArray)
- Conclusion: mNGS has high sensitivity and specificity for S. pneumoniae identification

<table>
<thead>
<tr>
<th>Table 3</th>
<th>Diagnostic performance of mNGS compared with culture and Alere BinaxNow® Streptococcus pneumoniae Antigen test for the detection of Streptococcus pneumoniae</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clinical microbiology tests</td>
<td>mNGS</td>
</tr>
<tr>
<td></td>
<td>Sensitivity (%)</td>
</tr>
<tr>
<td>Culture</td>
<td>73.1</td>
</tr>
<tr>
<td>Combined test</td>
<td>70.3</td>
</tr>
</tbody>
</table>

Note: a refers to the blood and/or CSF culture; b refers to culture and or Alere BinaxNow® Streptococcus pneumoniae Antigen test. PPV positive predictive value, NPV negative predictive value
Costs and economics

- Despite dropping costs MGx still costly and additive!
- Real life costs significantly higher (repeats, depth, batching)
- Costs go beyond just the sequencing
- Need costing models that include capital investment, personnel
- Medical reimbursement schemes
- And what about cost-effectiveness? Impact on QALY?
- Will MGx offset some existing costs?
- Worsening of disparities, esp. LMICs
Costs and economics

**Diagnostic Metagenomics List**

- NextSeq/HiSeq (+1 backup): $250k
- Initial validation costs: $120k
- Server/AWS time/storage: $40k
- 2 FTE MLS wet-lab: $150k/yr
- 1.5 FTE bioinformatician: $150k/yr
- 1 lab director (0.1 FTE): $10k/yr
- Library prep/QC reagents: $45k/yr
- Sequencing reagents (1 run/wk): $80-120k/yr
Costs and economics

In general, we find the use of metagenomic testing as second-line investigation is effectively dominated, and that use of metagenomic testing at first-line would typically require higher rates of detection or lower cost than currently available in order to be justifiable purely as a cost-saving measure.

We conclude that current conditions do not warrant a widespread rush to deploy metagenomic testing to resolve any and all uncertainty, but rather as a front-line technology that should be used in specific contexts, as a supplement to rather than a replacement for careful clinical judgement.
Human resource

- Worsening shortage of lab personnel
- Most cMGx work is not automated
- High demand for bioinformaticians (1:3-1:4)
- Commercial software is not a magic solution
- Budget cuts, efficiency
QC / QA

• No agreed quality assurance framework
• How to perform validation? All sample types? All pathogens?
• How to determine LOD / LOQ?
• Need calibrators, CRMs
• PPV/NPV of cMGx extremely important
• How to control cMGx over time?
• Some initial EQA efforts – mixed results
Regulation and accreditation

• Most countries still do not have regulatory frameworks for integrating WGS...
• International standard lacking – ISO etc.
• How to perform accreditation? nationally and internationally
• What needs to be validated or accredited and how?
• Legal aspects (e.g. lawsuits, liability)
THANK YOU