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Gut microbiota predicts severity and reveals novel metabolic signatures in acute pancreatitis

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ABSTRACT

Objective Early disease prediction is challenging in acute pancreatitis (AP). Here, we prospectively investigate whether the microbiome predicts severity of AP (Pancreatitis—Microbiome As Predictor of Severity; P-MAPS) early at hospital admission.

Design Buccal and rectal microbial swabs were collected from 424 patients with AP within 72 hours of hospital admission in 15 European centres. All samples were sequenced by full-length 16S rRNA and metagenomic sequencing using Oxford Nanopore Technologies. Primary endpoint was the association of the orointestinal microbiome with the revised Atlanta classification (RAC). Secondary endpoints were mortality, length of hospital stay and severity (organ failure >48 hours and/or occurrence of pancreatic collections requiring intervention) as post hoc analysis. Multivariate analysis was conducted from normalised microbial and corresponding clinical data to build classifiers for predicting severity. For functional profiling, gene set enrichment analysis (GSEA) was performed and normalised enrichment scores calculated.

Results After data processing, 411 buccal and 391 rectal samples were analysed. The intestinal microbiome significantly differed for the RAC (Bray-Curtis, p value=0.009), mortality (Bray-Curtis, p value 0.006), length of hospital stay (Bray-Curtis, p=0.009) and severity (Bray-Curtis, p value=0.008). A classifier for severity with 16 different species and systemic inflammatory response syndrome achieved an area under the receiving operating characteristic (AUROC) of 85%, a positive predictive value of 67% and a negative predictive value of 94% outperforming established severity scores. GSEA revealed functional pathway units suggesting elevated short-chain fatty acid (SCFA) production in severe AP.

Conclusions The orointestinal microbiome predicts clinical hallmark features of AP, and SCFAs may be used for future diagnostic and therapeutic concepts.

Trial registration number NCT04777812.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ The intestinal microbiome is altered in patients with acute pancreatitis (AP) compared with healthy controls.
⇒ Early prediction of disease severity in AP is challenging and often requires longitudinal data assessment.

WHAT THIS STUDY ADDS

⇒ Full-length 16S rRNA and metagenomic sequencing reveals significant alterations of the orointestinal microbiome in patients with AP that are associated with disease severity and clinical hallmark features such as length of hospital stay and mortality.
⇒ A classifier with 16 differentially abundant species and systemic inflammatory response syndrome outperforms established severity scores for AP.
⇒ All abundant species in severe AP belong to taxonomic families, which are known as common producers of short-chain fatty acids (SCFAs) and functional profiling suggest elevated SCFA production in severe AP.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ Implementation of a fast-track workflow is feasible for rapid, point-of-care microbiome diagnostics for patients with AP in the emergency department.
⇒ Our data may explain the failure of SCFA-containing probiotics in a clinical trial of predicted severe AP.
⇒ Exploration of microbiome-derived metabolic pathways and metabolites (eg, SCFA) in patients with AP might open new avenues for early and goal-directed treatment approaches.

INTRODUCTION

Acute pancreatitis (AP) is a major cause of hospital admission and results in a considerable socioeconomic burden.[1] The overall mortality is reported with 2% as most patients experience a mild course of disease. However, 10%–20% develop a moderate to severe course of disease with systemic and local complications. In particular, the combination of infected abdominal necrosis and organ failure such as respiratory, renal or circulatory failure dramatically increases the mortality rate.[2–4] Additionally, patients are also at increased risk to die within 90 days after hospital discharge.[5] Here, cardiovascular-related deaths contribute equally as AP-related infections. Besides elevated mortality, patients with severe AP experience major morbidity, decreased quality of life, long hospital stays of up to several months and often undergo multiple internal and external drainage procedures.[6 7]

To implement effective goal-directed and early treatment strategies, it is important to understand the molecular underpinnings of AP that determine the course of disease within the first few hours after hospital admission. Complex scoring systems such as the Ranson Score, APACHE II, Pancreatitis Activity Scoring System (PASS), Harmless Acute Pancreatitis Score (HAPS) and Bedside Index of Severity in Acute Pancreatitis (BISAP) were developed to predict severity of AP.[8–11] However, most of these scores are cumbersome to calculate and require longitudinal data assessment, rendering most classifier not feasible for daily clinical routine.[12] HAPS and BISAP are easy to assess but also result in unsatisfactory sensitivity and specificity around 70%. Presence or absence of systemic inflammatory response syndrome (SIRS) has also been proposed as marker for individual parameters of severity with a sufficient sensitivity and negative predictive value.[13 14] However, persistent SIRS occurs more often during or after persistent organ failure rather than before.[13] Thus, novel markers to reliably predict the course of disease early during hospital admission are needed.

Recently, the intriguing bilateral link between pancreatic diseases and the gut microbiome has attracted significant scientific and clinical attention.[15 16] To date, it is assumed that bacteria migrate to the pancreas in a retrograde fashion from the small bowel.[17 18] Interestingly, it was shown that healthy pancreas and pancreatic tumours harbour their own distinct intra-pancreatic microbiome.[17] Moreover, there is growing evidence that the intratumoral microbiome promotes tumour progression by altering the tumorous immune system.[17–19]

In AP, microbes translocate from the small bowel into pancreatic necrosis depending on activated regulatory T-cells.[20] Furthermore, it was postulated that a large proportion of patients suffer from an intestinal condition known as leaky gut that may play an important role in the pathogenesis of AP.[21] Here, it is believed that systemic inflammation and hypovolaemia lead to an increased translocation of bacteria from the intestines. In the PROPATRIA trial, Besselink *et al* aimed to ameliorate severe AP by administering probiotics to patients with predicted severe AP. Notably, the trial had to be discontinued after an interim analysis revealed an increased risk of mortality in the probiotic arm,[22] indicating the potential role of intestinal microbiota in the pathogenesis of AP.

To this end, it is not surprising that patients with AP have an altered intestinal microbiome compared with healthy controls.[23 24] Furthermore, preliminary studies with less than 60 patients investigated the rectal microbiome as biomarker for a necrotic course of disease and for the occurrence of respiratory distress syndrome.[25 26] However, there is a lack of comprehensive prospective clinical data investigating the role of the orointestinal microbiome in AP and its association with clinical hallmark features. Here, we conducted a prospective cohort study enrolling patients with AP from 15 European centres. The oral and rectal microbiome was analysed by full-length 16S rRNA gene and metagenomics sequencing at admission and associated with the revised Atlanta classification (RAC), mortality and length of hospital stay.[27]

METHODS

Recruitment and endpoints

For this European-wide, multicentric, prospective observational cohort study (Pancreatitis—Microbiome as Predictor of Severity; P-MAPS), 450 patients with acute pancreatitis (AP) were recruited from 15 European centres within 72 hours of hospital admission (online supplemental table S1). No transferred patients from other hospitals were included in the study. Sample size was calculated prior enrolment by power calculation (supplementary methods).[27] Patients were enrolled between March 2020 and June 2022. AP was diagnosed if two of the following criteria were fulfilled: (1) lipase ≥3 times of the upper limit, (2) characteristic upper abdominal pain, (3) imaging features in line with AP. After informed consent was obtained, buccal and rectal swabs (eSwab, Copan, Brescia, Italy) were collected according to previously published protocols.[28] All samples were frozen at −80°C within 1 hour after collection. Frozen swabs were shipped from external centres to University Medical Centre Goettingen on dry ice. Exclusion criteria were patients <18 years, pregnancy and imaging features or clinical signs of chronic pancreatitis. Clinical data of each patient were pseudonymously entered in an online database (SoSci Survey) (figure 1). The study was registered at clinical.trial.gov (NCT04777812). It was not possible to involve patients or the public in the design, or conduct, or reporting or dissemination plans of our study.

The primary endpoint is the association of the orointestinal microbiome with the RAC. Secondary endpoints are the association of orointestinal, microbiome with mortality, number of intervention and length of hospital stay.[27] Metadata for numbers of interventions were insufficiently obtained and thus it was not possible to associate the microbiome with this secondary endpoint. Severity of AP (organ failure >48 hours and/or the occurrence of pancreatic collections that required drainage) was defined as post hoc variable.

DNA extraction, sequencing, classification

A comprehensive wet-bench and bioinformatical workflow for analysing microbiome samples sequenced with Oxford Nanopore Technologies (ONT) was previously published by our group.[28] Detailed descriptions are provided in online supplemental methods. All buccal samples underwent full-length 16S rRNA gene sequencing and rectal samples were sequenced with the whole metagenomic approach. The previously established and validated MetaPont pipeline was used to classify microbial data.[28] All fastq files were uploaded in Qiita (study ID 15088) and the European Nucleotide Archive (ERP153335) in a per sample manner with their corresponding sample data and prep-data.[29] Functional profiling was assessed with DIAMOND and Megan6 Ultimate Edition (online supplemental methods).

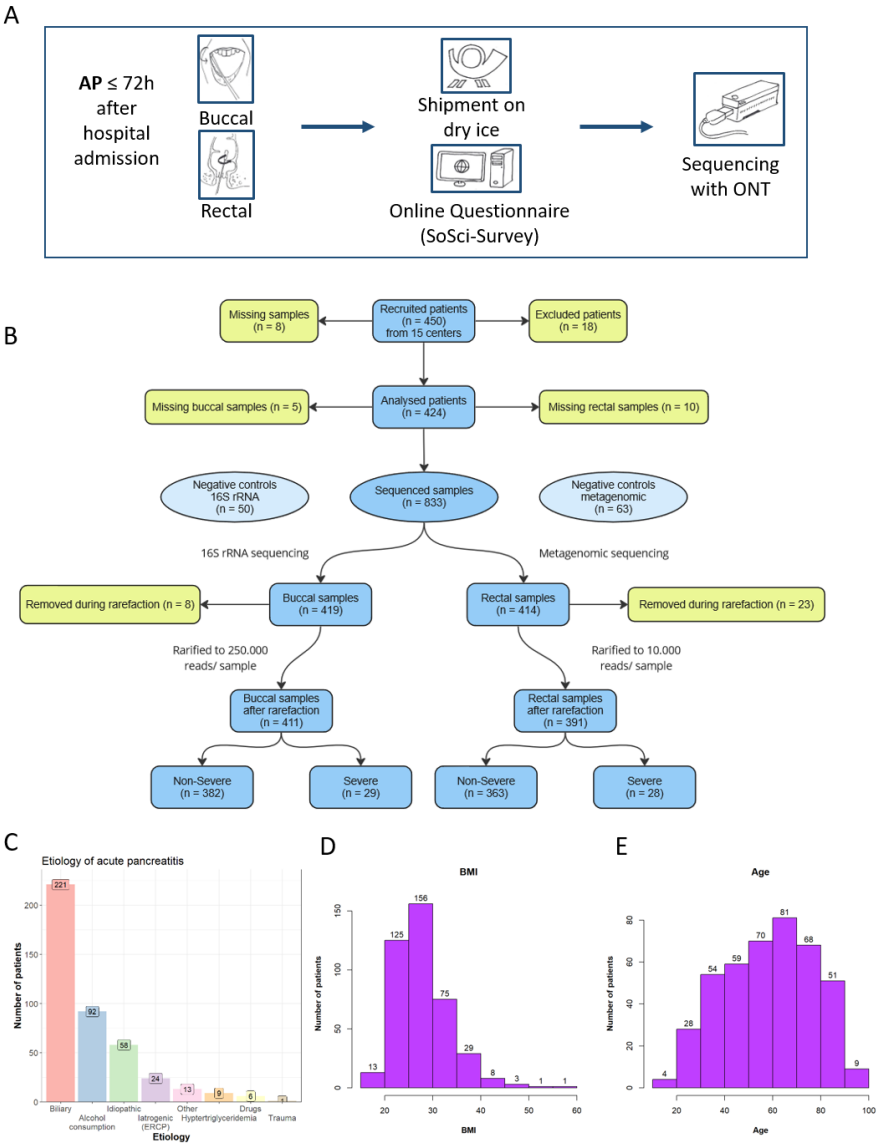

Figure 1 Study protocol and study population. (A) Patients with acute pancreatitis (AP) were recruited within 72 hours after hospital admission. Buccal and rectal swabs were stored at −80°C and were shipped on dry ice. Metadata were stored in SoSci Survey. All samples were sequenced using the Oxford Nanopore Technologies (ONT) platform. (B) Study population and negative control overview. Bar plots for (C) aetiologies, (D) body mass index (BMI) and (E) age. ERCP, endoscopic retrograde cholangiopancreatography.

Statistical analysis

All subsequent analyses were conducted in R V.4.1.2 or newer. Microbial data preprocessing is described in depth in online supplemental methods. After testing normality with Shapiro-Wilk test and testing homogeneity of variance by applying Levene test, a t-test or Mann-Whitney U test was performed, respectively. For variables with more than two groups, analysis of variance (ANOVA) or Kruskal-Wallis was applied depending on normal distribution. Details about alpha and beta-diversity metrics are implemented in supplementary methods. Whenever feasible, 95% CIs are provided for estimates. Results were regarded as significant with a two-sided p value <0.05. If more than three pairwise groups are compared, p values were adjusted for multiple testing. A detailed description of factoring in confounders is provided in online supplemental materials.

Multivariate analysis

Normalised microbial and corresponding clinical data were used to build classifiers for predicting severity for metagenomically sequenced rectal samples. A matched subgroup of a non-severe AP group was defined (non-severe, severe ratio 2:1) for identifying differential abundant species between severity groups (online supplemental methods). Extended and circumscribed classifiers were built with weighted regularised regression and random forest. More details are provided in online supplemental methods.

Differentially abundant species and SIRS were used to predict severity in a Ridge regression for the whole study population and compared with BISAP and HAPS. All receiver operating characteristics (ROC) for regression models were calculated by using leave-one out cross validation. Area under the receiving operating characteristics (AUROCs) for random forests are based on predicted case probabilities and reported without cross-validation.

RESULTS

Description of study population

In total, 450 patients were prospectively enrolled in the online database SoSci Survey. After revision of metadata 18 patients with signs of chronic pancreatitis (CP) were excluded. Moreover, samples from eight patients went missing during shipment to the University Medical Center Goettingen. To this end, 424 patients with corresponding 419 buccal and 414 rectal samples remained for sequencing (5 buccal and 10 rectal swabs were missing). After normalisation of the microbial data, 411 buccal and 391 rectal samples remained for subsequent analysis (figure 1B). Among eight different causes of AP, biliary was the most common followed by alcohol and idiopathic (figure 1C). Other causes for AP are listed in online supplemental tables S2 and S3. The median body mass index (BMI) was 26.9 kg/m^2 (figure 1D) and the median age was 60 years (figure 1E).

RAC is associated with early alterations of rectal microbiome

The RAC is a widely used classification of AP and subsumes three categories (RAC I–III).[30] The primary endpoint was associated with buccal and rectal microbiomes by calculating alpha-diversity and beta-diversity. To investigate, whether significant results derived from RACI-III, 79 potential confounding clinical features were factored in (online supplemental figure S1).

Interestingly, we did not observe any significant differences in buccal samples regarding α-diversity and β-diversity (online supplemental figure S2A–D). Accordingly, α-diversity indices were also not significant for rectal samples (online supplemental figure S2E). However, Bray-Curtis distance revealed a different microbial signature of RAC III compared with RAC I and RAC II, respectively (p values RAC I vs III=0.024*, RAC II vs III=0.009**), whereas the latter two almost completely overlapped in PCoA (p value RAC I vs II=1) (figure 2A). Differential abundance calculations with microbiome multivariable association with linear models 2 (MaAsLin2), linear discriminant analysis effect size (LEfSe) and other distance metrics strongly support this finding (figure 2B, online supplemental figure S2F–I). All three RAC subgroups differed regarding seven potential confounding factors (online supplemental table S4A,B). Five of these factors had an impact in microbial composition and thus were included in stratified PERMOANOVA (online supplemental figure S3A–C). However, our results remained highly significant (p value RAC III vs I and II=0.001**) (online supplemental table S5).

Mortality is associated with early alterations of rectal microbiome

Secondary endpoints were associated with buccal and rectal microbiomes by calculating alpha-diversity and beta-diversity. To investigate whether significant results derived from endpoints, 79 potential confounding clinical features were factored in (online supplemental figure S1).

In total, 10 patients of 424 died within 30 days after AP diagnosis or during the hospital stay. Notably, deceased patients revealed significantly less observed species in rectal (p value=0.041*) but not in buccal samples (p value=0.452) (online supplemental figure S4A,B). Both alpha-diversity metrics that emphasised evenness (Shannon, inverse Simpson index) remained insignificant in buccal and rectal samples (online supplemental figure S4A,B). However, the rectal but not buccal microbiome was significantly different between alive and deceased patients regarding Bray-Curtis distances (p value=0.006**), other beta-diversity indices and differential abundances (figure 2C,D, online supplemental figure S4C–I). Patients who died within 30 days were significantly older (p value=0.026*) and had a lower BMI (p value=0.037*) compared with survivors (online supplemental figure S5A,B). Notably, both groups did not significantly differ regarding other clinical features (online supplemental table S6A,B). Pairwise distance comparison for both groups separately indicated that age and BMI had an impact on microbial composition and thus were incorporated in stratified PERMANOVA test for Bray-Curtis metrics (online supplemental figure S5C,D). However, stratification of PERMANOVA still provided a significant result (p value=0.013*) (online supplemental table S5).

Length of hospital stay is associated with early alterations of rectal microbiome

Before analysing the association of microbial data with length of hospital stay, all deceased patients were excluded. To this end, 401 buccal and 381 rectal samples were included. A weak but significant negative correlation was calculated between alpha-diversity (observed species) and length of hospital stay in buccal and rectal samples (buccal: p value=0.01*, Rho=−0.13, rectal: p value=0.049*, Rho=−0.1), but remained insignificant for Shannon and Inverse Simpson Index (online supplemental figure S6A–F). Bray-Curtis distances showed significant differences in PERMANOVA test (p value=0.009**) for rectal samples (figure 2E). Other beta-diversity metrics for buccal and rectal samples except weighted UniFrac (UF) also showed significant changes (online supplemental figure S7A–F). Differential abundance calculation revealed significant differences of species between short (<30 days) and long hospital duration (≥30 days) (figure 2F, online supplemental figure S7G). We further investigated potential confounding variables (online supplemental table S7A,B; online supplemental figure S8A–K). A stratified PERMANOVA confirmed that Bray-Curtis distances were significant (p value=0.007**) (online supplemental table S5).

Post hoc definition of severe versus non-severe acute pancreatitis

Severe pancreatitis was defined as having persistent organ failure (>48 hours) following AP and/or the occurrence of pancreatic collections that required drainage (figure 3A). This post hoc endpoint was chosen since the majority of Atlanta II patients (n=87) did not require interventional drainage of necrotic collections and could be discharged significantly earlier compared with Atlanta II patients that required interventional drainage (n=6; 13.4 days vs 24.2 days; p=0.003**). Thirty out of 424 patients were considered as severe AP according to these criteria (table 1). After normalisation of microbial data, 29 buccal and 28 rectal samples from severe AP, and 382 buccal and 363 rectal samples for non-severe AP were subsequently analysed (figure 3A). Overall, the 30-day or in-hospital mortality was 2.4%. However, patients with severe AP showed a higher mortality (26.67% vs 0.51 %, p value <0.0001***) compared with the non-severe group (figure 3B). SIRS was defined when two or more of four criteria were present and occurred significantly more often in the severe AP group (p value <0.0001***) (table 1). Established prediction scores like the BISAP and HAPS score significantly higher in the severe group (both p values<0.0001***) (figure 3C,D). Furthermore, patients with severe AP stayed significantly longer in hospital than the non-severe AP group (p value <0.0001***) (figure 3E). Severe AP

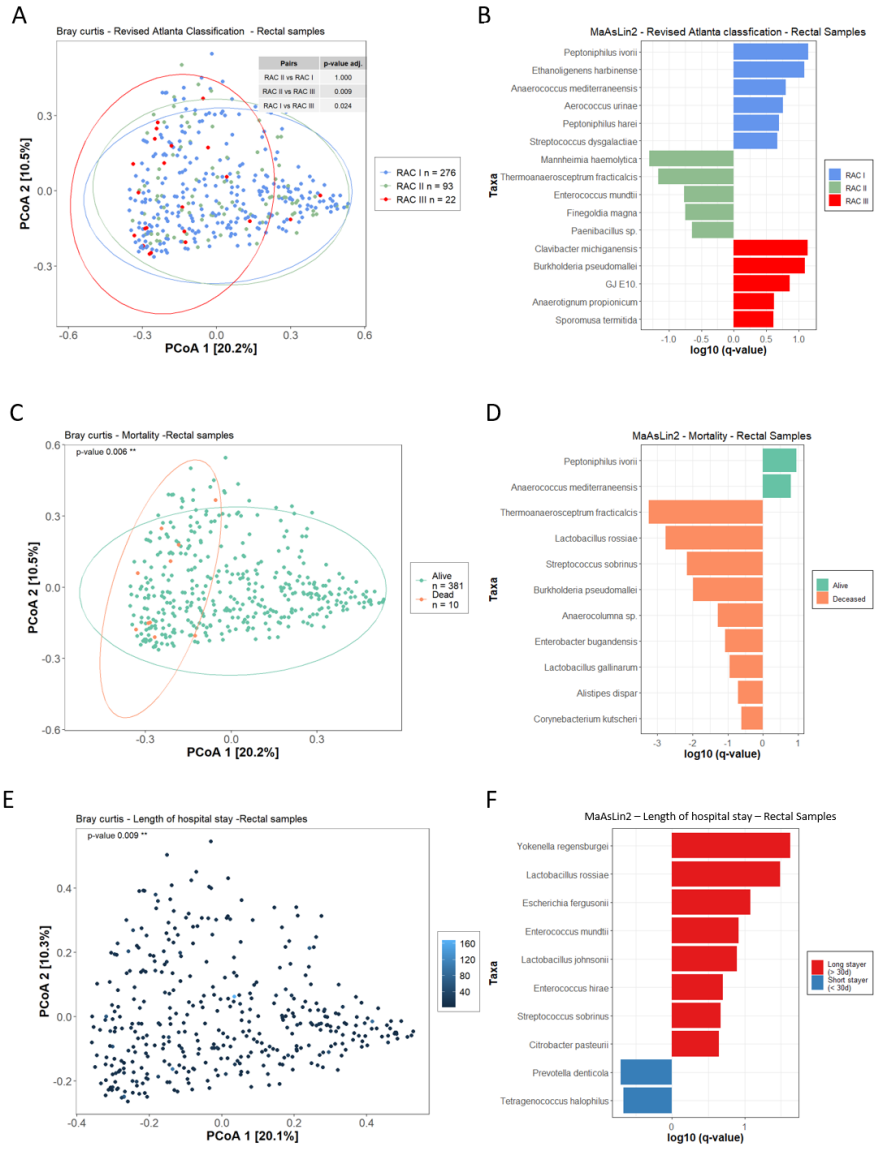

Figure 2 Association of rectal microbiome with primary and secondary endpoints. (A) Bray-Curtis distances were plotted in PCoA for rectal samples and were grouped for revised Atlanta classification (RAC I=blue, RAC II=green, RAC III=red). (B) Differential abundances between RAC subgroups were calculated with MaAsLin2 and displayed in bar plots. Rectal samples were associated with mortality with (C) Bray-Curtis distances and (D) differential abundances (alive=light green, dead=orange). (E) The β-diversity distances for rectal microbiome were continuously coloured for length of hospital stay. (F) For differential abundances, a cut-off of 30 days was chosen and patients were grouped accordingly (long stayer ≥30 days=red, short stayer <30 days=blue). P values for β-diversity were calculated by PERMANOVA. Length of hospital stay was rank-transformed for PERMANOVA tests. For MaAsLin2, all potential confounders were included in multivariable testing and species were considered as differentially abundant if q-value <0.25. MaAsLin2, microbiome multivariable association with linear models; PCoA, principal coordinate analysis.

was also accompanied by more frequent and higher grades of organ failure, more frequent intensive care unit (ICU) admissions, and higher frequencies of necrotic AP and infected collections (table 1).

Disease severity is associated with microbial shift in rectal microbiome

Three different indices were calculated to obtain α-diversity. All indices did not show any significant differences between groups in buccal and rectal samples (online supplemental figure S9A,B). Regarding β-diversity, Bray-Curtis distance metrics were significantly different between severe and non-severe AP for rectal (p value=0.008**) but not buccal swabs (p value=0.571) (figure 3F, online supplemental figure S10A–C). Notably, these results were confirmed with other β-diversity distance metrics (online supplemental figure S10D–F). Moreover, differential abundances obtained by MaAslin2 and LEfSe revealed several differentially abundant species in both groups (figure 3G, online supplemental figure S10G). It is commonly known that the microbiome is influenced by several host dependent and independent factors.[31] Again, we tested whether 79 known clinical features as potential confounding variables have an impact on microbial composition and thus explain the microbial shift more than severity. For severity, both groups did not differ significantly in any potential confounding variable (online supplemental table

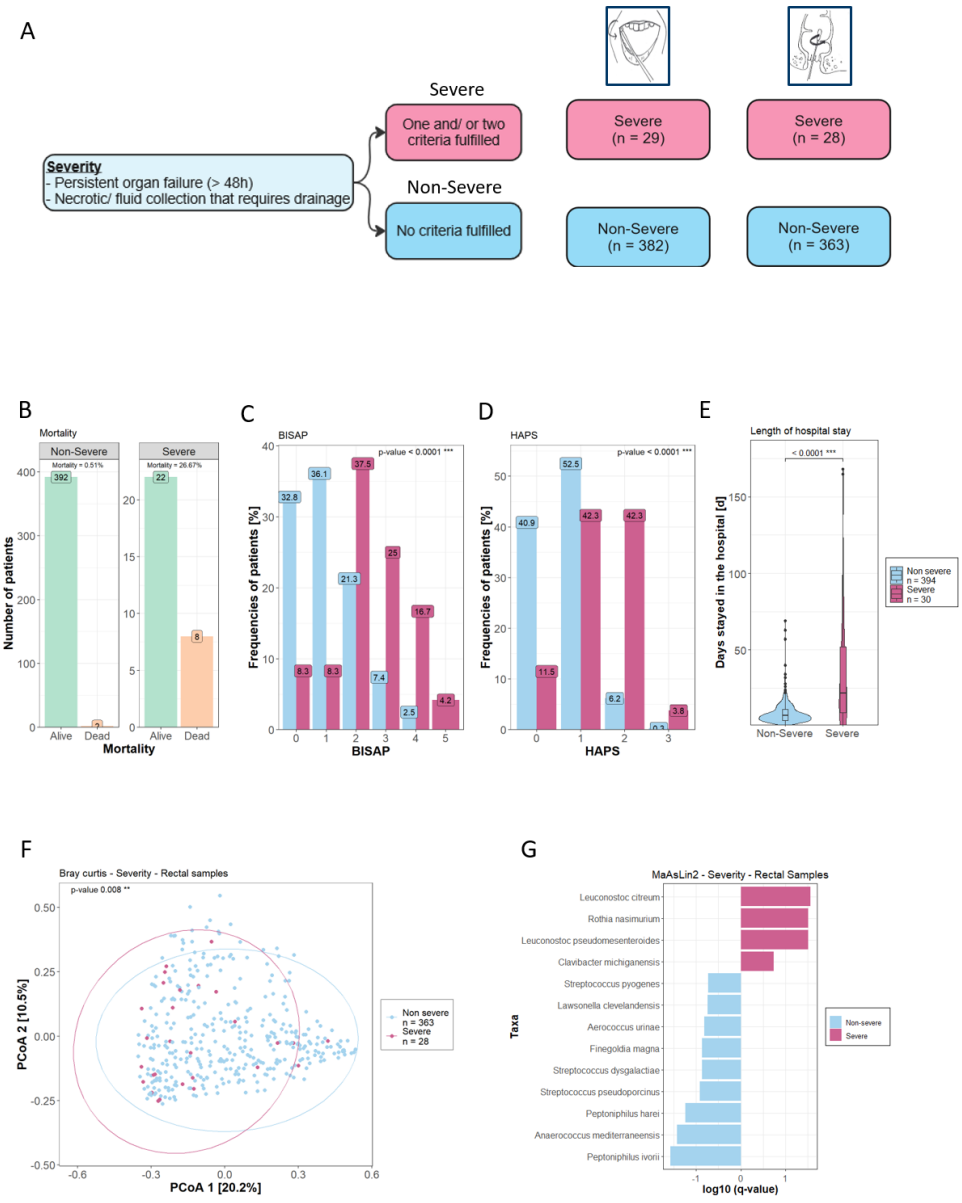

Figure 3 Association of rectal microbiome data with severity. (A) Definition and sample size of severe (violet) and non-severe (light blue) for buccal and rectal samples. (B) Mortality in severe and non-severe patients. Bar plots showing distributions of severe and non-severe APs regarding (C) BISAP score, (D) HAPS and (E) a violin plot for length of hospital stay. (F) For β-diversity, Bray-Curtis distances are ordinated with PCoA for rectal swabs. PERMANOVA was used to test significance. (G) Differential abundances between non-severe and severe were calculated with MaAsLin2 (including all potential confounding variables q-value <0.25) and displayed in bar plots. BISAP, Bedside Index of Severity in Acute Pancreatitis; HAPS, Harmless Acute Pancreatitis Score; LEfSe, linear discriminant analysis effect size; MaAsLin2, microbiome multivariable association with linear models; PCoA, principal coordinate analysis.

S8A,B). Notably, severity remained significant in permutated ANOVA for distance-based redundancy analysis (db-RDA) for rectal samples even if ten frequently reported confounding variables were incorporated in db-RDA (p value=0.022*) (online supplemental figure S10H, online supplemental table S9). However, the biplot of db-RDA revealed that vectors of some variables heading in the same or the opposite direction as severity, and thus can bias the PERMANOVA results. To address this issue, we stratified the PERMONOVA test for these variables and yielded a significant difference between severe and non-severe APs (p value=0.013*, online supplemental table S5). Moreover, focusing on R^2 values obtained by PERMANOVA test revealed that severity only accounts for a moderate variance compared with other confounding factors like country from where the sample originated (online supplemental figure S11A).

Matched cohorts identify 16 differentially abundant species in severe versus non-severe AP

To identify species that are more directly linked to disease severity, we matched patients based on possible confounding features and then repeated the procedure. To this end, we diminished the influence of potential confounding variables on microbial composition by extracting a subpopulation from the non-severe group that matched with the severe group with a target ratio of 2:1 (figure 4A). The matching was stratified for country, antibiotic intake and gender (online supplemental

Table 1 Complications in severe and non-severe patients

Variable	Non-severe (n=394)	Severe (n=30)	P value	Significance
Acute fluid collection	72 (18.3%)	11 (36.7%)	0.028	*
Acute kidney injury, KDIGO I	23 (5.8%)	0 (0%)	0.393	ns
Acute kidney injury, KDIGO II	4 (1.0%)	11 (36.7%)	<0.0001	***
Acute kidney injury, KDIGO III	1 (0.3%)	9 (30.0%)	<0.0001	***
ANC	21 (5.3%)	10 (33.3%)	<0.0001	***
Acute respiratory failure grade 1*	0 (0%)	1 (3.3%)	0.071	ns
Acute respiratory failure grade 2*	1 (0.3%)	0 (0%)	1	ns
Acute respiratory failure grade 3*	0 (0%)	4 (13.3%)	<0.0001	***
Acute respiratory failure grade 4*	0 (0%)	0 (0%)		
Acute respiratory failure invasive ventilation	0 (0%)	11 (36.7%)	<0.0001	***
Acute respiratory failure non-invasive ventilation	1 (0.3%)	1 (3.3%)	0.137	ns
Cardiovascular failure grade 1†	1 (0.3%)	6 (20.0%)	<0.0001	***
Cardiovascular failure grade 2†	0 (0%)	0 (0%)		
Cardiovascular failure grade 3†	0 (0%)	1 (3.3%)	0.071	ns
Cardiovascular failure grade 4†	2 (0.5%)	8 (26.7%)	<0.0001	***
Death	2 (0.5%)	8 (26.7%)	<0.0001	***
Drainage requirement	0 (0%)	16 (53.3%)	<0.0001	***
Duodenal obstruction	6 (1.5%)	6 (20.0%)	<0.0001	***
ERCP requirement	66 (16.8%)	4 (13.3%)	0.801	ns
Haematological failure	0 (0%)	1 (3.3%)	0.071	ns
Hepatic failure	0 (0%)	0 (0%)		
ICU admission	11 (2.8%)	16 (53.3%)	<0.0001	***
Infected collection	4 (1.0%)	10 (33.3%)	<0.0001	***
Mesenterial thrombosis	6 (1.5%)	4 (13.3%)	0.003	**
Necrotic course (WON or ANC)	26 (6.6%)	14 (46.7%)	<0.0001	***
Neurological failure	0 (0%)	1 (3.3%)	0.071	ns
No complication	277 (70.3%)	0 (0%)	<0.0001	***
Other organ failure	1 (0.3%)	3 (10.0%)	0.001	**
Pseudocyst	16 (4.1%)	3 (10.0%)	0.143	ns
SIRS‡	75 (21.7%)	22 (81.5%)	<0.0001	***
WON	5 (1.3%)	7 (23.3%)	<0.0001	***

*Grade 1: PaO_2/FIO_2=400–301, grade 2: PaO_2/FIO_2=300–201, grade 3: PaO_2/FIO_2=200–101, grade 4: PaO_2/FIO_2 <101.
†Grade 1: Systolic blood pressure <90 mm Hg not responsive to fluid resuscitation; grade 2: systolic blood pressure <90 mm Hg, pH <7.3; grade 3: systolic blood pressure <90 mm Hg, pH <7.2; grade 4: catecholamine requirement
‡SIRS was available for 345 non-severe and 27 severe APs.
ANC, acute necrotic collection; AP, acute pancreatitis; ERCP, endoscopic retrograde cholangiopancreaticography; ICU, intensive care unit; KDIGO, Kidney Disease: Improving Global Outcomes; SIRS, systemic inflammatory response syndrome; WON, walled-off necrosis.

table S10). After matching rarified samples, 28 severe and 53 non-severe remained for further analysis (25 severe matched to two non-severe, each; three severe matched to one corresponding non-severe, because of stratification). The Bray-Curtis distance remained significant (p value=0.001***) between severe and non-severe AP, and both groups were more separated compared with the whole population (figure 4B). Also, the R^2 value of severity increased 10-fold and was the only significant variable in PERMANOVA test besides RAC (online supplemental figure S11B). Taken together, this matched population showed clearer shifts of microbial compositions between severe and non-severe AP than the total study population. Consequently, we calculated differential abundances with this respective cohort. There are several approaches to obtain differential abundances with different underlying statistical approaches.[32 33] MaAslin2 is based on generalised linear and mixed model and allows multivariate analysis.[34] Here, all potential confounding variables are included in this multivariable analysis. LEfSe is a widely applied method, applicable to rarefied microbial features using non-parametric tests and subsequently linear discriminant analysis (LDA).[35] In total, 18 species with MaAsLin2 (q value<0.05) and 51 species with LEfSe (LDA score >2, p value <0.05) were defined as differential abundant. Furthermore, we applied a median abundance filter (figure 4C). Sixteen species had a proportion above this filter of 0.002 in at least one group and were considered as relevant for being included in the circumscribed classifier (figure 4D).

Sixteen differentially abundant species and SIRS can predict disease severity in AP

To predict severity, an extended and a circumscribed classifier were built with regularised regressions and random forest. First, to assess the concept, we used an extended classifier to predict severity for the matched population. Here, all 819 species and 79 clinical features (metadata) were included in an elastic net regularised regression yielding an AUROC of 77.6% (figure 4E). Further results from regression and random forest extended classifiers are described in the supplementary results (online supplemental table S12A,B). Notably, the extended classifier with all rectal species is able to predict severity. Next, we

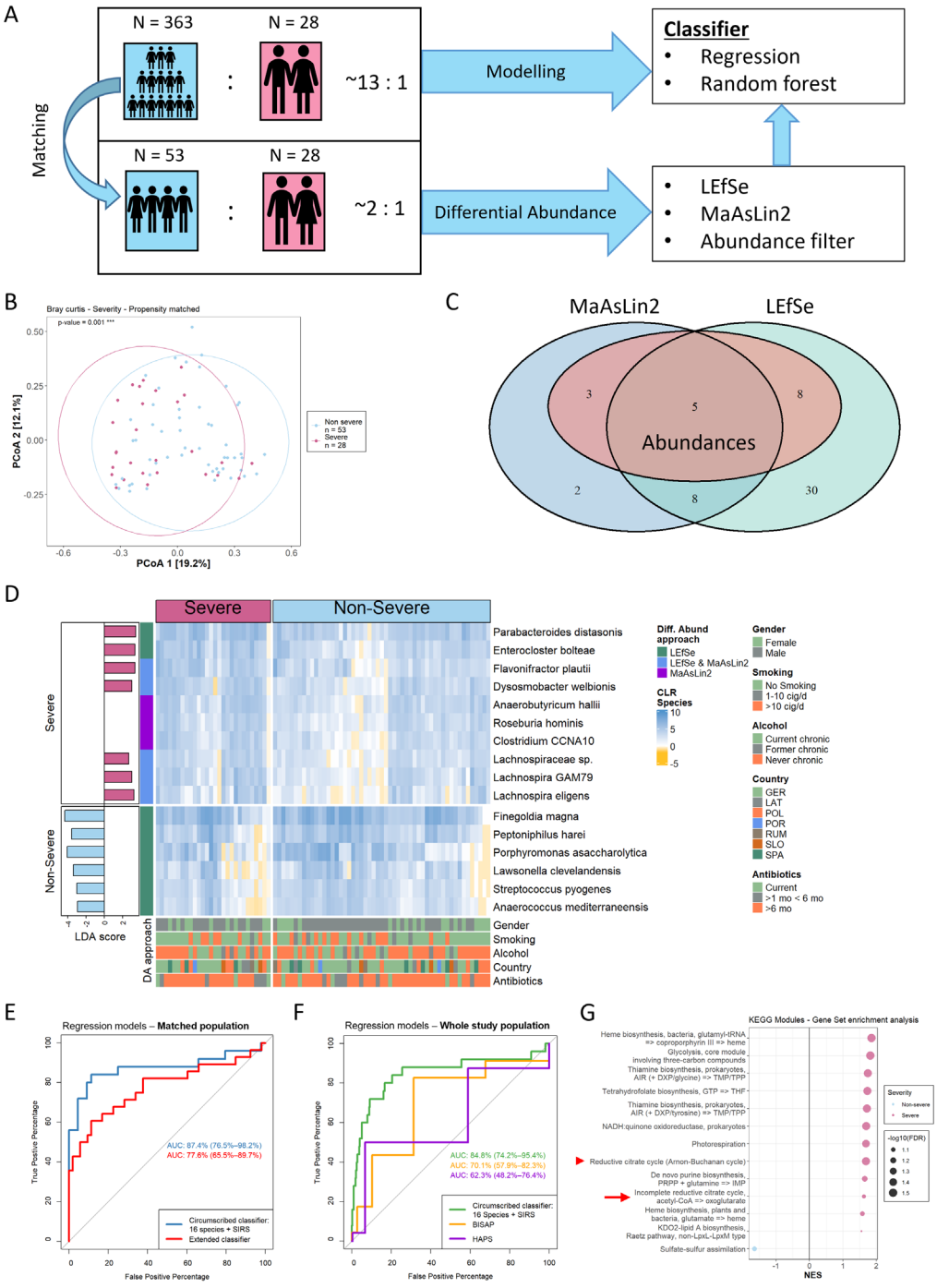

Figure 4 Building classifiers for prediction of severity. (A) The graphical summary describes the modelling process. By using the matchIT package, 53 patients were identified from the non-severe cohort who matched best regarding 79 clinical features. (B) Bray-Curtis distances were calculated for rectal samples of matched population and PERMANOVA test was performed to assess p value. (C) The Venn diagram explains the distribution of differential abundant species obtained by LEfSe (LDA score >2, p value < 0.05), MaAsLin2 (all potential confounders included and q value <0.05) and abundance filter (median proportion 0.002 in at least one group). (D) A heatmap displays the distribution of centred log transformed (CLR) abundances of 16 differential abundant species and clinical parameters in matched study population. An extended (elastic net) and circumscribed (Ridge) model was built for (E) matched cohort and (F) whole study population. The extended model included all 819 rectal species and all 79 potential clinical confounders. For circumscribed model, 16 differential abundant species and SIRS were combined and were compared with BISAP and HAPS. (G) Gene set enrichment analysis (GSEA) of KEEG orthologies (KOs) calculated for KEGG modules revealed functional pathway units which contribute to short-chain fatty acid (SCFA) production (red arrows) more expressed in severe APs (violet). AP, acute pancreatitis; BISAP, Bedside Index of Severity in Acute Pancreatitis; HAPS, Harmless Acute Pancreatitis Score; LEfSe, linear discriminant analysis effect size; LDA, linear discriminant analysis; MaAsLin2, multivariable association with linear models 2.

constructed a circumscribed model with 16 differential abundant species and systemic inflammatory response syndrome (SIRS). SIRS was chosen because of the easy assessment and the known predictive value.[13] SIRS alone is not highly sensitive to determine persistent organ failure in AP.[36] However, in combination with further clinical features, it is part of established risk scores like BISAP.[11] Notably, our combined circumscribed regression approach achieved an AUROC of 87.4% in regression and in random forest 88. 6% (figure 4E, online supplemental table S12A,B). Finally, we rebuild the circumscribed classifiers on the whole study population. Here, the 16 differential abundant species and SIRS (non-severe n=317 and severe n=25) still yielded an AUROC of 84.8% in Ridge regression, outperforming established scores such as BISAP (70.1%) (non-severe n=223 and severe n=23) and HAPS (62.3%) (non-severe n=309 and severe n=24) (figure 4F). AUROC of 16 species was 62.4% and for SIRS alone 77.7% (online supplemental figure S10I). Most remarkably, these 16 differential abundant species and SIRS yielded a positive predictive value of 66.6%, a negative predictive value of 94% and an accuracy of 93.3%. To this end, we conclude that rectal species in combination with SIRS predict severity better than BISAP and HAPS.

Functional profiling identifies SCFA producing pathways in severe AP

Interestingly, all differentially abundant species over-represented in severe AP (*Parabacteroides distasonis*, *Enterocloster bolteae*, *Dysosmobacter welbionis*, *Flavonifractor plautii*, *Lachnospira GAM79*, *Lachnospiraceae* sp., *Lachnospira eligens*, *Roseburia hominis*, *Anaerobutyricum hallii* and *Clostridium CCNA10*) belong to taxonomic families, which are widely recognised as common producers of SCFAs.[37–39] Therefore, we analysed rectal samples from all matched patients (severe n=28, non-severe n=53) regarding their functional profiles. For GSEA, two different gene background lists were created: (1) KEGG orthologies (KOs) cluster for KEGG pathway (n=452) and (2) KOs cluster for KEGG modules (n=477). Interestingly, GSEA of KOs calculated for KEGG modules revealed functional pathway units which contribute to SCFA production to be more expressed in severe APs compared with non-severe (figure 4G, online supplemental figure S12 and online supplemental material). All count-tables KEGG orthologies per sample are publicly available.[40]

DISCUSSION

Alterations of the human microbiome have been linked to a variety of inflammatory conditions. Here, we aim to explore associations between the microbiome and clinically relevant parameters in the early phase of AP. To this end, we prospectively enrolled 450 patients in 8 European countries from 15 centres to evaluate the oral and intestinal microbiome by full-length 16S rRNA and metagenomic sequencing. Our microbial data convincingly show for the first time that the orointestinal microbiome is associated with established parameters of severity in AP.

Stool is widely used to investigate the gut microbiome and was considered as gold standard.[41] However, we decided to use rectal swabs to determine intestinal microbial compositions in patients with AP. The timing was crucial for sample collection as our aim was to assess the microbiome within 72 hours of admission. Most patients with AP suffer from severe pain and frequently require opioids that contribute to bowel paralysis in the early phase rendering sample collection unreliable in the 72-hour timeframe. Previous studies exploring the intestinal microbiome in AP also used rectal swabs.[25 26] Furthermore, due to the oxygen gradient, there are different horizontal niches of the gut microbiomes. Therefore, the microbiome derived from rectal swabs is more similar to biopsy-derived microbiome than to faeces.[42] Accordingly, rectal swabs harbour more microbes adjacent to the mucosa compared with stool samples that represent more the anaerobic luminal microbiota.

Notably, our microbial data from rectal swabs were sequenced with a whole metagenomic approach, whereas the buccal swabs were analysed using a full-length 16S rRNA approach due to the lower biomass that did not reliably yield sufficient sequencing depths. The major advantage of both approaches for long-read based sequencing is resolution at species level and consequently a more profound microbial analysis.[43] In contrast, existing studies only investigated dysbiosis between healthy subjects and patients with AP, included less than 60 patients and used short-read 16S rRNA sequencing describing microbiome alterations at genus or higher taxonomic ranks.[23 25 26]

Regarding our study population from eight European countries, the age distribution with a peak between 50 and 60 years as well as the overall mortality of ~2% are comparable to previously published epidemiological data.[44] Also in line with previous reports, gallstones (52.1%) and alcohol use (21.7%) were the most common causes of AP followed by idiopathic AP with 13.6%.[44] Since the RAC does not distinguish whether pancreatic fluid collections require interventional drainage, the majority of RAC II patients had a relatively mild course of disease with organ failure <48 hours mostly due to existing comorbidities and self-limiting fluid collections. In contrast, a smaller subgroup within RAC II required interventional drainage and had a significantly prolonged hospital stay prompting us to define severe AP with organ failure (>48 hours) and/or the occurrence of pancreatic collections that required interventional therapy. On the contrary, patients with pancreatic collection without the need for drainage and absence of persistent organ failure were considered as non-severe AP.

Importantly, our microbial data demonstrate that alterations of the microbiome are associated with the RAC, disease severity, mortality and length of hospital stay. However, an association of the microbiome with clinical endpoints can be confounded by multiple internal and external factors such as gender, previous medication and pre-existing diseases[31] that are sometimes not sufficiently accounted for in major microbiome studies.[45] Thus, we carefully collected and factored in multiple known confounding features using a comprehensive approach which was previously applied by our group.[46] Using this approach, we have not observed any significant differences regarding 79 potential confounding factors between severe and non-severe AP. In addition, to further reduce the impact of these confounding factors on microbial composition and to identify differentially abundant species which most likely explain the observed differences between severe and non-severe AP, we extracted a subpopulation from non-severe AP that matched best to the smaller severe AP group. Differential abundance calculation of this matched cohort identified 16 intestinal species that could be applied to build a classifier for severity on the whole population. This classifier outperformed widely used scoring systems such as BISAP, HAPS and SIRS. Notably, the combination of SIRS and 16 differentially abundant species yielded the best discriminator between patients with severe and non-severe AP. Importantly, clinical score assessment and microbial sampling should be performed in parallel to avoid biases introduced by delayed microbial sampling. Although this is currently the largest cohort for microbiome analysis in the context of AP, the sample size of

severe patients (n=28) remains relatively small. Consequently, future studies are required to validate the performance of the respective classifier.

Unexpectedly, all differentially abundant species in severe AP belong to families which are recognised as common producers of SFCAs,[37–39] and subsequent GSEA revealed functional pathway units contributing for SCFA production. In contrast to our findings, SCFAs are widely considered for having beneficial effects in multiple diseases, including ameliorating acute pancreatitis.[47–49] One explanation could be the fact that previous translational animal studies examined the luminal microbiome from stool samples. As rectal swabs represent more the mucosa-adherent microbiome, the differences observed in our study might the consequence of niche-specific changes on severe AP. This spatial variation of dysbiosis is a common phenomenon in other intestinal microbiome alternating diseases.[50 51] However, there is evidence that SCFA producing species and strains also might be harmful. For instance, it was shown that they contribute to disease progression in metabolic diseases and inflammatory bowel diseases due to an excess of propionate.[52 53] Furthermore, the PROPATRIA trial that examined the prophylactic effect of probiotics in predicted patients with severe AP had to be stopped after interim analysis since mortality was increased in the probiotic arm.[22] Intriguingly, the probiotic formula applied in this trial consisted of six SCFA-producing species.[54] Currently, we can only speculate whether SCFA producing bacteria are cause or consequence during the early phase of severe AP. Thus, it would be interesting to explore the dynamics and function of SCFA-producing species and targeted metabolomics during the onset of severe AP. Possibly, this knowledge could guide novel diagnostic, therapeutic and preventive concepts and clinical trials in the future.

Currently, our workflow from sample collection, DNA extraction, sequencing and bioinformatical analysis takes up to 4 days and limits the utility of a point-of-care diagnostics in clinical routine. However, we have already tested a fast-track workflow that would enable us to obtain reliable microbiome data from buccal and rectal swabs within few hours. This fast-track approach could pave the way for interventional clinical studies that are urgently needed to improve the individual management and overall outcome of patients with AP.

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Contributors CA-H, AN, KLA, TA: Study design, data analysis and interpretation. CA-H, GB, SMB, SC, GC, MD, FF, AG, RH, AH, CM, LN, VN, AP, CP, VP, AP, SR, DR, ER, MLR-R, SS, MW, SV, JW: Patient enrolment and sample collection. LD, CA-H, JH: Bioinformatics and functional profiling. CA-H and VE: Funding; CA-H and AN: Writing of manuscript. All authors revised and approved the final version of the manuscript. AN is the guarantor of the study.

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REFERENCES

1 Peery AF, Crockett SD, Barritt AS, *et al*. Burden of gastrointestinal, liver, and pancreatic diseases in the United States. *Gastroenterology* 2015;149:1731–41.
2 Vege SS, Gardner TB, Chari ST, *et al*. Low mortality and high morbidity in severe acute pancreatitis without organ failure: a case for revising the Atlanta classification to include "moderately severe acute pancreatitis" *Am J Gastroenterol* 2009;104:710–5.
3 Mofidi R, Duff MD, Wigmore SJ, *et al*. Association between early systemic inflammatory response, severity of multiorgan dysfunction and death in acute Pancreatitis. *Br J Surg* 2006;93:738–44.
4 Kwong W-Y, Ondrejková A, Vege SS. Predictors and outcomes of moderately severe acute pancreatitis - evidence to reclassify. *Pancreatology* 2016;16:940–5.
5 Czapári D, Váradi A, Farkas N, *et al*. Detailed characteristics of post-discharge mortality in acute pancreatitis. *Gastroenterology* 2023;165:682–95.

6 Pendharkar SA, Salt K, Plank LD, *et al*. Quality of life after acute pancreatitis: a systematic review and meta-analysis. *Pancreas* 2014;43:1194–200.
7 Gliem N, Ammer-Herrmenau C, Ellenrieder V, *et al*. Management of severe acute pancreatitis: an update. *Digestion* 2021;102:503–7.
8 Ranson JH, Rifkind KM, Roses DF, *et al*. Prognostic signs and the role of operative management in acute pancreatitis. *Surg Gynecol Obstet* 1974;139:69–81.
9 Lankisch PG, Weber-Dany B, Hebel K, *et al*. The harmless acute pancreatitis score: a clinical algorithm for rapid initial stratification of nonsevere disease. *Clin Gastroenterol Hepatol* 2009;7:702–5;
10 Lankisch PG, Warnecke B, Bruns D, *et al*. The APACHE II score is unreliable to diagnose necrotizing pancreatitis on admission to hospital. *Pancreas* 2002;24:217–22.
11 Wu BU, Johannes RS, Sun X, *et al*. The early prediction of mortality in acute pancreatitis: a large population-based study. *Gut* 2008;57:1698–703.
12 Capurso G, Ponz de Leon Pisani R, Lauri G, *et al*. Clinical usefulness of scoring systems to predict severe acute pancreatitis: a systematic review and meta-analysis with pre and post-test probability assessment. *United European Gastroenterol J* 2023;11:825–36.
13 Singh VK, Wu BU, Bollen TL, *et al*. Early systemic inflammatory response syndrome is associated with severe acute pancreatitis. *Clin Gastroenterol Hepatol* 2009;7:1247–51.
14 Kumar A, Chari ST, Vege SS. Can the time course of systemic inflammatory response syndrome score predict future organ failure in acute pancreatitis *Pancreas* 2014;43:1101–5.
15 Ammer-Herrmenau C, Pfisterer N, Weingarten MF, *et al*. The microbiome in pancreatic diseases: recent advances and future perspectives. *United European Gastroenterol J* 2020;8:878–85.
16 Frost F, Kacprowski T, Rühlemann M, *et al*. Impaired exocrine pancreatic function associates with changes in intestinal microbiota composition and diversity. *Gastroenterology* 2019;156:1010–5.
17 Pushalkar S, Hundeyin M, Daley D, *et al*. The pancreatic cancer microbiome promotes oncogenesis by induction of innate and adaptive immune suppression. *Cancer Discov* 2018;8:403–16.
18 Aykut B, Pushalkar S, Chen R, *et al*. The fungal mycobiome promotes pancreatic oncogenesis via activation of MBL. *Nature* 2019;574:264–7.
19 Geller LT, Barzily-Rokni M, Danino T, *et al*. Potential role of Intratumor bacteria in mediating tumor resistance to the chemotherapeutic drug Gemcitabine. *Science* 2017;357:1156–60.
20 Glaubitz J, Wilden A, Frost F, *et al*. Activated regulatory T-cells promote duodenal bacterial translocation into necrotic areas in severe acute Pancreatitis. *Gut* 2023;72:1355–69.
21 Wu LM, Sankaran SJ, Plank LD, *et al*. Meta-analysis of gut barrier dysfunction in patients with acute pancreatitis. *Br J Surg* 2014;101:1644–56.
22 Besselink MG, van Santvoort HC, Buskens E, *et al*. Probiotic prophylaxis in predicted severe acute pancreatitis: a randomised, double-blind, placebo-controlled trial. *Lancet* 2008;371:651–9.
23 Zhang XM, Zhang ZY, Zhang CH, *et al*. Intestinal microbial community differs between acute pancreatitis patients and healthy volunteers. *Biomed Environ Sci* 2018;31:81–6.
24 Zhu Y, He C, Li X, *et al*. Gut Microbiota dysbiosis worsens the severity of acute pancreatitis in patients and mice. *J Gastroenterol* 2019;54:347–58.
25 Zou M, Yang Z, Fan Y, *et al*. Gut Microbiota on admission as predictive biomarker for acute necrotizing pancreatitis. *Front Immunol* 2022;13:988326.
26 Hu X, Han Z, Zhou R, *et al*. Altered gut Microbiota in the early stage of acute pancreatitis were related to the occurrence of acute respiratory distress syndrome. *Front Cell Infect Microbiol* 2023;13:1127369.
27 Ammer-Herrmenau C, Asendorf T, Beyer G, *et al*. Study protocol P-MAPS: Microbiome as predictor of severity in acute pancreatitis-a prospective multicentre translational study. *BMC Gastroenterol* 2021;21:304.
28 Ammer-Herrmenau C, Pfisterer N, van den Berg T, *et al*. Comprehensive wet-bench and bioinformatics workflow for complex Microbiota using Oxford nanopore Technologies. *mSystems* 2021;6:e00750-21.
29 Ammer-Herrmenau C, Neesse A. Fastq-files: Qiita (study ID 15088). n.d. Available: https://qiita.ucsd.edu/study/list/
30 Banks PA, Bollen TL, Dervenis C, *et al*. Classification of acute pancreatitis--2012: revision of the Atlanta classification and definitions by international consensus. *Gut* 2013;62:102–11.
31 Vujkovic-Cvijin I, Sklar J, Jiang L, *et al*. Host variables confound gut Microbiota studies of human disease. *Nature* 2020;587:448–54.
32 Lin H, Peddada SD. Analysis of microbial compositions: a review of normalization and differential abundance analysis. *NPJ Biofilms Microbiomes* 2020;6:60.
33 Yang L, Chen J. A comprehensive evaluation of microbial differential abundance analysis methods: current status and potential solutions. *Microbiome* 2022;10:130.
34 Mallick H, Rahnavard A, McIver LJ, *et al*. Multivariable Association discovery in population-scale meta-Omics studies. *PLoS Comput Biol* 2021;17:e1009442.
35 Segata N, Izard J, Waldron L, *et al*. Metagenomic biomarker discovery and explanation. *Genome Biol* 2011;12:R60.
36 Mounzer R, Langmead CJ, Wu BU, *et al*. Comparison of existing clinical scoring systems to predict persistent organ failure in patients with acute Pancreatitis. *Gastroenterology* 2012;142:1476–82;
37 Awoniyi M, Wang J, Ngo B, *et al*. Protective and aggressive bacterial subsets and metabolites modify hepatobiliary inflammation and fibrosis in a murine model of PSC. *Gut* 2023;72:671–85.
38 Nogal A, Valdes AM, Menni C. The role of short-chain fatty acids in the interplay between gut Microbiota and diet in Cardio-metabolic health. *Gut Microbes* 2021;13:1–24.
39 Wang K, Liao M, Zhou N, *et al*. Parabacteroides distasonis alleviates obesity and metabolic dysfunctions via production of succinate and secondary bile acids. *Cell Rep* 2019;26:222–35.
40 Ammer-Herrmenau C, Neesse A. Count-tables KEGG-Orthologies per sample. n.d. Available: https://owncloud.gwdg.de/index.php/s/m0jIaTIIVFKPw32
41 Knight R, Vrbanac A, Taylor BC, *et al*. Best practices for analysing microbiomes. *Nat Rev Microbiol* 2018;16:410–22.
42 Albenberg L, Esipova TV, Judge CP, *et al*. Correlation between Intraluminal oxygen gradient and radial partitioning of intestinal microbiota. *Gastroenterology* 2014;147:1055–63.
43 Johnson JS, Spakowicz DJ, Hong B-Y, *et al*. Evaluation of 16S rRNA gene sequencing for species and strain-level microbiome analysis. *Nat Commun* 2019;10:5029.
44 Lankisch PG, Apte M, Banks PA. Acute pancreatitis. *Lancet* 2015;386:85–96.
45 Forslund K, Hildebrand F, Nielsen T, *et al*. Disentangling type 2 diabetes and metformin treatment signatures in the human gut Microbiota. *Nature* 2015;528:262–6.
46 Ammer-Herrmenau C, Lingens CHM, Ratei CS, *et al*. Whole 16S rRNA sequencing of the oral Microbiome predicts postoperative pancreatic fistula: prospective observational cohort study. *Br J Surg* 2023;110:1279–83.
47 Vacca M, Celano G, Calabrese FM, *et al*. The controversial role of human gut lachnospiraceae. *Microorganisms* 2020;8:573.
48 Pan X, Fang X, Wang F, *et al*. Butyrate ameliorates Caerulein-induced acute pancreatitis and associated intestinal injury by tissue-specific mechanisms. *Br J Pharmacol* 2019;176:4446–61.
49 van den Berg FF, van Dalen D, Hyoju SK, *et al*. Western-type diet influences mortality from Necrotising Pancreatitis and demonstrates a central role for butyrate. *Gut* 2021;70:915–27.
50 Yang M, Hong G, Jin Y, *et al*. Mucosal-associated microbiota other than luminal microbiota has a close relationship with diarrhea-predominant irritable bowel syndrome. *Front Cell Infect Microbiol* 2020;10:515614.
51 Lavelle A, Lennon G, O'Sullivan O, *et al*. Spatial variation of the colonic microbiota in patients with ulcerative colitis and control volunteers. *Gut* 2015;64:1553–61.
52 Png CW, Lindén SK, Gilshenan KS, *et al*. Mucolytic bacteria with increased prevalence in IBD mucosa augment in vitro utilization of Mucin by other bacteria. *Am J Gastroenterol* 2010;105:2420–8.
53 Sanna S, van Zuydam NR, Mahajan A, *et al*. Causal relationships among the gut microbiome, short-chain fatty acids and metabolic diseases. *Nat Genet* 2019;51:600–5.
54 LeBlanc JG, Chain F, Martín R, *et al*. Beneficial effects on host energy metabolism of short-chain fatty acids and vitamins produced by Commensal and Probiotic bacteria. *Microb Cell Fact* 2017;16:79.