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Surface proteome of plasma extracellular vesicles differentiates between SARS-CoV-2 and influenza infection

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ABSTRACT

Small extracellular vesicles (sEVs) play a role in the pathophysiology of viral respiratory infections and may be suitable biomarkers for COVID-19 and Influenza infections, or targets for treatment. We investigated differences in the surface proteome of plasma sEVs in patients with COVID-19 and Influenza. In a discovery cohort with 117 patients, we used a random forest (RF) classifier in order to discriminate COVID-19 and Influenza patients based on routine clinical parameters. Furthermore, plasma samples from these patients were analyzed with an EV Array containing 33 antibodies to capture sEVs, which were then visualized with a combination of CD9, CD63, and CD81 antibodies. We applied an RF classifier and a random depth-first search (RDFS) approach to extract markers with the best discriminatory potential. Data were then validated in an independent set of patient samples on a chip-based ExoView platform.In the initial cohort of 117 patients, leukocyte numbers, and heart rate discriminated best between COVID-19 and Influenza infection. In the plasma samples, 32 EV surface markers could be detected. Feature panels containing CD9, CD81, and CD141 allowed a discrimination between COVID-19 and Influenza. Consecutively, increased CD9 abundance was validated in a second, independent cohort, with the ExoView technology. The increased CD9 signal in Influenza patients was confirmed and shown to be mostly driven by CD9/CD41a double positive sEVs, hinting at a thrombocyte origin.We identified leukocyte numbers and heart rate, as well as CD9 as a sEV surface marker to differentiate COVID-19 from Influenza patients.

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Introduction

The coronavirus disease 2019 (COVID-19) pandemic, caused by severe acute respiratory syndrome-related coronavirus 2 (SARS-CoV-2), has resulted in tremendous morbidity, mortality, and socio-economical disturbance. As of May 2023, there have been almost $8x10^8$ confirmed global cases of COVID-19, and almost 7 million fatalities (https://covid19.who.int/). Pending many unresolved clinical and pathophysiological issues, severity of disease seems to be paralleled by an imbalance between interferon mechanisms [1] and neutrophil activation [2], as well as pro-coagulant events [3].

Coronaviruses are a group of single-stranded positive-sense RNA viruses. An infection with the new SARS-CoV-2 is characterized by a range of symptoms including fever, dry cough, and general malaise [4]. Patients with severe COVID-19 develop acute respiratory distress syndrome and acute lung injury, leading to increased morbidity and mortality caused by damage to the alveolar tissue, leading to inflammation and pneumonia [5].

The co-occurrence of COVID-19 and Influenza, another seasonal RNA virus of the airways, poses a potential global health burden, even though it is presently a rare event [6]. While surveillance and vaccination are paramount to public

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healthcare [7], early correct diagnosis upon a patient's presentation in the clinic is another building block of a conclusive healthcare [8]. This co-occurrence of Influenza A virus with SARS-CoV-2 requires timely distinction between both diseases in the clinic. Patients that are hospitalized routinely undergo screening of a multitude of health-relevant biological parameters. We address the value of these parameters for early differentiation between COVID-19 and Influenza. Furthermore, soluble, blood-borne small extracellular vesicles (sEVs) have emerged as important players in various physiological and pathological processes, including viral infections [9]. In the context of COVID-19 disease caused by the SARS-CoV-2 virus, there is growing evidence that sEVs released from infected cells play a crucial role in the pathogenesis of the disease [10]. sEVs contain a variety of bioactive molecules, including proteins, nucleic acids, and lipids, which can be transferred to recipient cells and modulate their function. Studies have shown that sEVs released from SARS-CoV-2 infected cells can contribute to the systemic inflammation, cytokine storm, and endothelial dysfunction observed in severe COVID-19 cases [11]. Moreover, sEVs may serve as potential biomarkers for disease diagnosis, prognosis, and as therapeutic targets. Understanding the role of sEVs in COVID-19 disease may offer new insights into the pathogenesis of the disease and pave the way for the development of new diagnostic and therapeutic strategies. The surface protein decoration of these sEVs is subject to alteration, dependent on the state of their cell of origin. It has previously been published that circulating EVs from SARS-CoV-2 patients have increased pro-coagulant activity when compared to healthy donors [12]. This suggests that sEVs are subject to change in the wake of an infection.

We hypothesize that SARS-CoV-2 and the subsequent immune response shape the composition of EV surface marker proteins in a specific way that allows discrimination of a SARS-CoV-2 infection from other infectious diseases of the lung. We therefore investigated the sEV surface proteome of patients suffering from SARS-CoV-2 or Influenza infection in two independent cohorts and different methodology. We could show that more CD9 positive sEVs exist in plasma samples of Influenza patients compared to plasma of COVID-19 patients, and that they are probably of thromobocytic origin.

Methods

Patient samples

Initial plasma samples from patients with acute COVID-19 (n = 100) and Influenza (n = 17) (Table 1) in our initial cohort were obtained from the German Competence Network of community acquired pneumonia (CAPNETZ) [13]. Equally, plasma samples were obtained from the validation cohort (COVID-19 n = 8, Influenza n = 6, Table S1). Plasma samples and clinical data were accessed through a research project submitted to the CAPNETZ STIFTUNG (application number: 2020–04-23-P9CM). CAPNETZ inclusion criteria are fulfillment of one or more of the following criteria: Cough, purulent sputum, positive auscultation findings, fever. Exclusion criteria are hospitalization more than 48 h prior to diagnosis of pneumonia or active pulmonary tuberculosis within the last 2 months. Of the 131 total patients, 127 have been recruited across Germany, while 4 have been recruited in Switzerland. All patients have been recruited before 23 March 2021. Therefore, no patient had received a COVID vaccination. Nineteen patients (4 Influenza patients and 15 COVID patients) had a seasonal Influenza vaccination in the past 13 months. The study was carried out following definitions of Good Clinical Practise, according to the declaration of Helsinki. Signed informed consent was obtained from every single individual for prospective biobanking. Institutional Review Board approval of every single center was obtained. A central IRB approval is available by the Ethics Committee of the Hannover Medical School; Project approval number: 301–2008. Routine parameters of the initial cohort were used for discriminating patients with acute COVID-19 from influenza infection. Where necessary, units of clinical parameters were converted for standardization purposes (Table S2).

Table 1. Patient characteristic of the study cohort (EV Array).

	COVID-19	Influenza
Patient Number	*n* = 100	*n* = 17
Age	55 (41, 68)	48 (38, 61)
Gender		
f	41 (45%)	6 (35%)
m	59 (55%)	11 (65%)
BMI	26.6 (24.0, 30.3)*	26.3 (23.5, 31.2)§
CRP[mg/l]	51 (19, 102)$	134 (44, 208)
ICU	8 (8%)	0 (0%)
Median (IQR)		

*Unknown = 8, $Unkown=2, §Unknown=2.

Ev antibody microarray

See supplementary methods. Data were deposited on the figshare database, DOI https://doi.org/10.6084/m9.figshare.28009130.

ExoView analysis

Plasma samples from the validation cohort were spun 2 times at 10,000 × g to clear potential contaminants. ExoView chips with CD9, CD63, and CD81

(tetraspanins), as well as CD41a capture antibodies were purchased from NanoView Biosciences and measured on an ExoView R100 imaging platform according to the manufacturer's protocol as described elsewhere [14]. Chips were pre-scanned before loading with patient plasma samples to establish a baseline for the measurement. CD9, CD63, and CD81 fluorescent antibodies included in the kit were used for detection.

Feature ranking

All steps are described in the supplementary methods and were implemented using MATLAB (R2020b, The MathWorks, Inc.) and its Statistics and Machine Learning Toolbox. As the SVMs with radial kernel performed best regarding reduced number of surface protein markers while maintaining a high performance assessed by the AUC, these ML models were used in the RDFS approach (Table S3).

To account for the imbalance between the COVID-19 ($n = 100$) and Influenza ($n = 17$) cohorts, we applied oversampling of the Influenza cases before train-validation split and model training (repeating the analysis with oversampling after train-validation split yielded similar results). This approach ensured a more balanced class distribution and prevented bias of the random forest classifier toward the majority (COVID-19) class. Influenza patients were randomly selected and duplicated until a total number of 50 Influenza patients was reached (new ratio: 2:1). After oversampling, the dataset contained 100 COVID-19 patients and 50 Influenza patients. Patient comorbidities (respiratory or pulmonary disease, vascular or cerebrovascular disease, chronic disease, malignant disease, other disease) were included in the model to assess their confounding potential.

Statistics

Statistical methods were employed as indicated. For analysis of the ExoView data, 2-way ANOVA was used. $p < 0.05$ was considered significant.

Results

Characterisation of the patient cohort

From a German multi-center discovery cohort (CAPNETZ), clinical and laboratory data and plasma samples from 100 COVID-19 patients and 17 Influenza patients were retrieved. Both patient groups were very similar in terms of age, gender, and BMI distribution (Table 1).

To create a benchmark for our surface marker analysis, we first assessed the discriminatory potential of routinely collected clinical and laboratory parameters in distinguishing COVID-19 from Influenza patients. In order to account for the heterogeneous nature of the routine parameters (different measurement scales), we employed a random forest (RF) classifier to identify parameters with discriminatory potential between COVID-19 and Influenza. The RF classifier was trained on 44 parameters, and its performance was assessed using 10-fold cross-validation (CV). The classifier achieved an excellent separation between COVID-19 and Influenza patients on both training (AUC: $1.00 \pm 0.42 \times 10^{-3}$) and validation data (AUC: $1.00 \pm 13.50 \times 10^{-3}$). Ranking diagnostic parameters by their importance for the RF classifier in distinguishing the two patient groups, we found leukocyte value, systolic blood pressure, and heart rate at 3 days post admission to be the three highest ranking diagnostic parameters (Figure 1(A)). While leukocyte values were significantly increased in Influenza patients vs. COVID-19 patients ($p < 0.001$), COVID-19 patients showed a significant increase in heart rate 3 days after admission vs. Influenza patients ($p < 0.001$, Figure 1(B)). Systolic blood pressure showed no significant difference between the two patient groups ($p > 0.05$, Figure 1(B)). From the remainder of the 10 highest ranking routine parameters (Figure 1(A)), the heart rate at admission ($p < 0.01$) and the thrombocyte values ($p < 0.05$) were significantly increased in COVID-19 compared to Influenza patients, while CRP was reduced ($p < 0.01$, Figure S1A). Comorbidities had very low importance values (respiratory or pulmonary disease: 0.31, vascular or cerebrovascular disease: 0.21, chronic disease: 0.22, malignant disease: 0.23, other disease: 0.23), highlighting their minor contribution to the model.

To further test which number of ranked routine parameters would be sufficient to distinguish COVID-19 from Influenza patients with a high accuracy, we trained RF classifiers on different subsets of routine parameters and compared their performance (Figure S1B). With the eight highest ranking parameters (out of 44), a high efficacy was reached on both the training and validation data, resulting in a very clear distinction between the two groups ($AUC_{training}$: $1.00 \pm 0.86 \times 10^{-3}$, $AUC_{validation}$: $0.99 \pm 16.46 \times 10^{-3}$). A subsequent addition of more routine parameters did not lead to a recognizable improvement.

When grouping COVID-19 by their ICU admission, COVID-19 ICU patients tended to be similar to Influenza patients (Figure S2). Notably, CRP levels were similar between COVID-19 ICU and Influenza

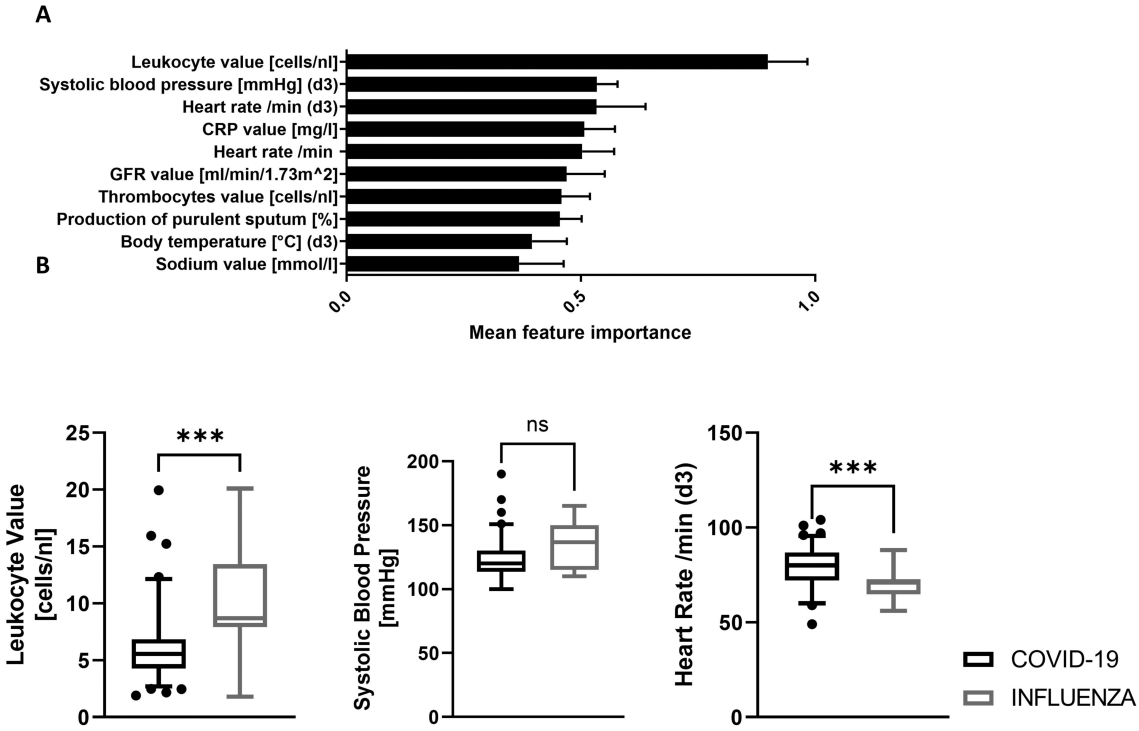

Figure 1. Identification of differential clinical diagnostic markers between COVID-19 and Influenza patients. (A) Routinely collected diagnostic clinical parameters were systematically investigated by a random-forest feature-selection approach. The top 10 clinical markers are shown, ranked by their mean feature importance + SD. (B) Differences between COVID-19 ($n = 100$) and Influenza ($n = 17$) patients for the three highest ranking clinical markers are shown. Data are shown as 25th to 75th percentiles (boxes) and 5th to 95th percentile (whiskers). The median is shown as a horizontal line. Statistical significance was assessed by Mann-Whitney Test. $^{**}p < 0.01$, $^{***}p < 0.001$.

patients, while they were significantly lower in COVID-19 patients who had not been admitted to ICU ($p < 0.001$). This pattern was also true for the leukocyte value ($p < 0.001$). In contrast, COVID-19 ICU patients were significantly different from Influenza patients in terms of thrombocytes value ($p < 0.001$), while non-ICU COVID-19 patients showed intermediate levels. Thus, the increased thrombocyte levels in COVID-19 patients were primarily driven by the subset of COVID-19 patients that were admitted to ICU.

Patient differentiation based on sEV surface marker proteins

In plasma samples from COVID-19 and Influenza patients, abundance data were obtained for 32 surface marker proteins. Two different feature selection methods were used to identify surface marker proteins that allowed discrimination between COVID-19 and influenza (Figure 2(A)). We first used the RF classifier which we also had employed for the clinical data in order to extract surface marker proteins with discriminatory potential. Using only EV surface marker proteins, the RF classifier reached a comparable performance ($AUC_{training}$: 1.00 ± 0.00, $AUC_{validation}$: $0.99 \pm 25.23 \times 10^{-3}$) to the 44 clinical parameters. Ranking the surface marker proteins by importance identified CD62E as the most prominent distinguishing feature, followed by ICAM-1 and CD41 (Figure 2(B)). These three surface protein markers together with CD151 (ranked fourth) were enough to distinguish between COVID-19 and Influenza patients with a performance comparable to all 32 markers ($AUC_{training}$: 1.00 ± 0.00, $AUC_{validation}$: $1.00 \pm 8.43 \times 10^{-3}$) (Figure S3). CD62E was significantly different between COVID-19 and Influenza patients ($p < .0.05$, Figure 2(C)).

We also tested the feature-selection approach RDFS, which selects subsets of features based on the performance of an SVM (Figure 2(A)). Ranking the sEV surface protein markers by their appearance in the selected subsets, the RDFS identified CD9, CD81, and CD141 as most frequently selected markers (Figure 2(D)). CD9 was not significantly different on sEVs from Influenza patients compared to sEVs from COVID-19 patients (Figure 2(E)). Furthermore, we tested CD63, CD81, and CD141. CD63 and CD81 only showed marginal differences on the EV Array (Figure S4),

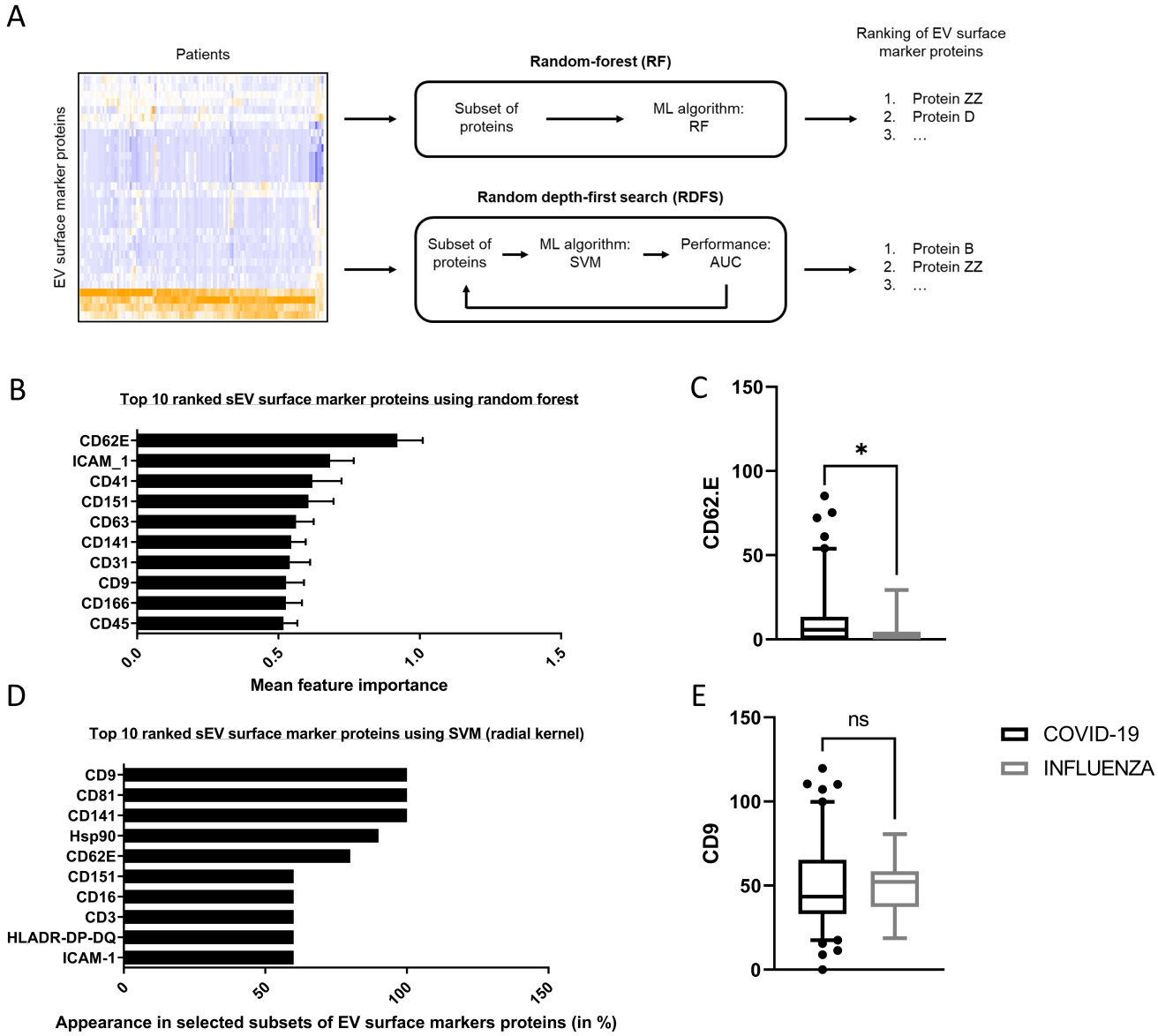

Figure 2. Identification of differential sEV marker panels between COVID-19 and Influenza patients. All 32 surface marker intensities from the EV microarray across 17 Influenza and 100 COVID-19 patients were systematically investigated by ev antibody microarray (a). Top 10 ranked EV surface marker proteins, selected by our feature-selection approach using random forest (b) or RDFS with SVM (d) are shown. The markers are ranked by their mean feature importance + SD (b) Or the number of appearance (in %) in the resulting subsets of features, which were selected by using a SVM with radial kernel (d). The top-listed marker (CD62E, C; CD9, e) is shown as 25th to 75th percentiles (boxes) and 5th to 95th percentile (whiskers). The median is shown as a horizontal line. Statistical significance was assessed by Mann-Whitney Test. *$p < 0.05$.

but we included them in the subsequent validation experiments, as they are all tetraspanins and classical EV marker proteins, such as CD9. When we stratified into ICU admission, the COVID-19 ICU patients differ significantly from non-ICU COVID-19 patients and Influenza patients in terms of CD9 ($p < 0.05$), CD62E ($p < 0.01$), CD63 ($p < 0.05$) and CD81 ($p < 0.01$) expression, suggesting their main potential in more severe COVID-19 cases (Figure S5). A combination of routine parameters and sEV markers as input for an RF classifier led to a similar performance ($AUC_{training}$: 1.00 ± 0.00, $AUC_{validation}$: $0.99 \pm 19.0 \times 10^{-3}$) compared to the two separate approaches described before. The 10 highest ranking parameters of this combined approach are shown in Figure S6.

Validation of sEV surface marker proteins in an independent validation cohort

We collected and measured eight new plasma samples from COVID-19 patients and six new plasma samples from Influenza patients (see Table S1 for patient

characteristics) on the ExoView platform. Combining the results from our feature rankings, we visualized CD9 and CD81, which were both in the top list of the RDFS approach (Figure 2(D)), and CD63 (found by RF, Figure 2(B)) on EVs that were captured by anti-CD41a, anti-CD9, anti-CD81, or anti-CD63 antibodies. We included surface markers that did not show strong differences on the original array as controls. The exemplary ExoView staining patterns for a sample from a COVID-19 patient and an Influenza patient are shown (Figure 3(A)). Significantly more particles were captured in Influenza samples, especially by the CD9 capture spot ($p < 0.001$, Figure 3(B)). While particle size was comparable between Influenza and COVID-19 samples (Figure 3(C)), the fluorescence indicative of CD9 was significantly increased on Influenza samples vs. COVID-19 samples ($p < 0.05$, Figure 3(D)). This confirmed the tendency that we already observed on the EV Array by median (Figure 2(E)).

Association between clinical parameters and CD9

As our validation data suggest CD9 as a differential marker between COVID-19 and Influenza samples, we tested the association of CD9 with the highest-ranking routine parameters from our RF approach (Figure 1(A)). We controlled for sex, age, and smoking status as potentially confounding variables. The only laboratory parameter with a significant association with CD9 was the thrombocyte value ($p < 0.05$, Table S4, Figure S7). Furthermore, comorbidities only correlated weakly with EV surface markers levels of CD9 ($r < 0.21$ in all cases), CD63 ($r < 0.12$ in all cases), and CD81 ($r < 0.12$ in all cases),

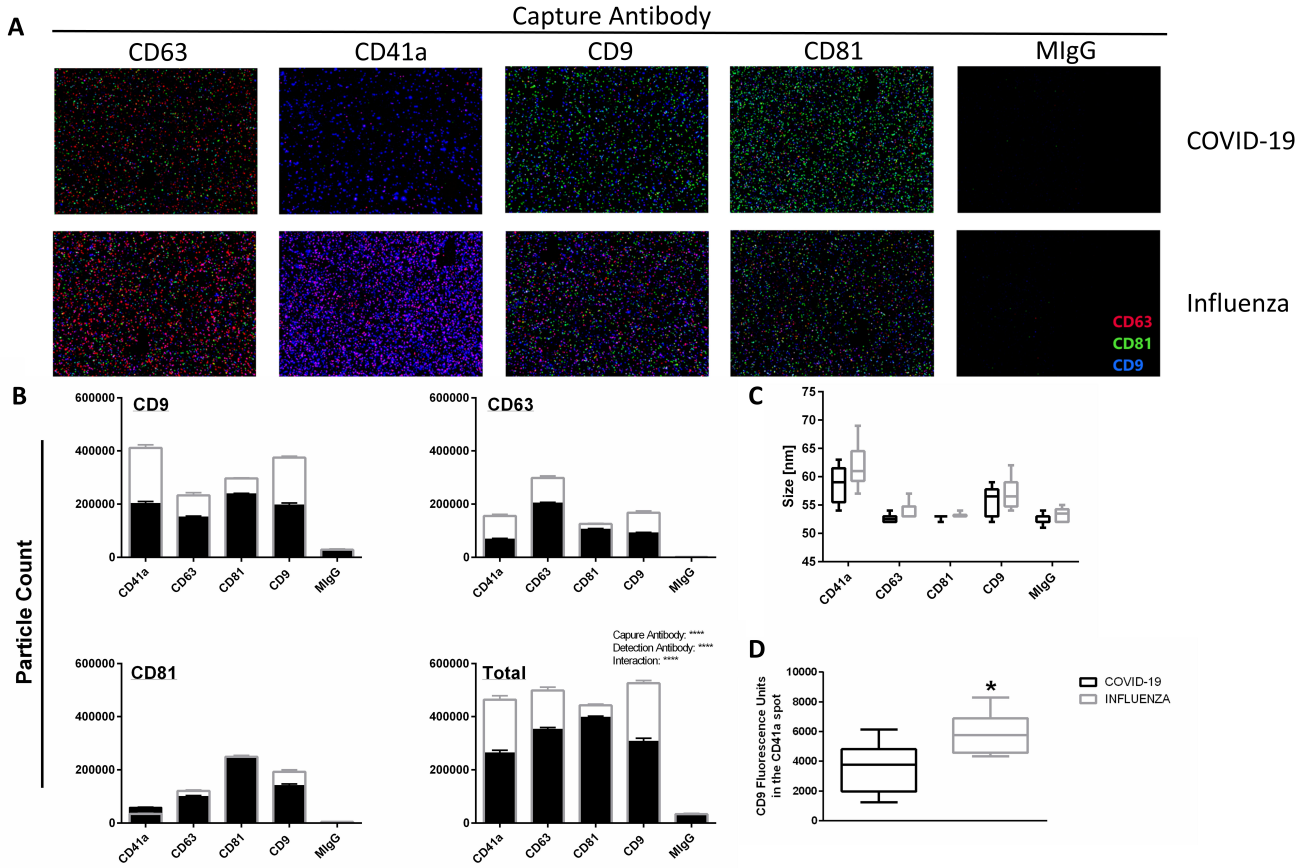

Figure 3. Validation of selected surface markers in a validation cohort by Exoview. COVID-19 ($n = 8$) and Influenza ($n = 6$) plasma samples were additionally measured as validation cohort on an ExoView platform to confirm selected markers from the protein array results. Exoview images are exemplary depicted of a COVID-19 and Influenza sample. Detection antibodies are indicated by color (a). Particle count distribution for each disease is shown as a function of capture antibody (x-axis) and detection antibody indicated per panel (mean count + SD is shown). Particle counts in Influenza samples were higher, especially on particles that stained CD9-positive (b), while vesicle size was equal between COVID-19 and Influenza patient samples (c). The fluorescence indicative of CD9 was significantly increased on Influenza samples vs. COVID-19 samples (d). Significance was assessed by 2-way ANOVA. MIgG served as isotype control. *$p < 0.05$, ****$p < 0.0001$.

Discussion

In this study, we analyzed routine clinical and laboratory parameters as well as the surface marker decoration of extracellular vesicles that we gained from the plasma of patients suffering from COVID-19 and Influenza. We extracted eight routine parameters by an RF approach that were, taken together, excellent discriminators between COVID-19 and Influenza. The discriminatory power of leukocyte value and thrombocyte value was sufficient to differentiate COVID-19 patients with ICU admission, COVID-19 patients without ICU admission, and Influenza patients (all without ICU admission). We furthermore identified a set of sEV surface molecules by applying two feature-selection approaches (RF or RDFS) that showed the potential to differentiate between COVID-19 and Influenza disease. CD9 was one of the most prominent sEV surface molecule that was found by both feature-selection approaches alike. We therefore chose to confirm CD9 as a discriminating sEV surface marker, and additionally measured CD81 and CD63, on an ExoView platform. We could show that the total particle count was higher in Influenza samples, especially when detecting CD9 positive vesicles. We could thereby corroborate CD9 as a valid marker to differentiate between vesicles from COVID-19 and Influenza patients. Furthermore, we show CD9, CD62E, CD63, and CD81 levels to be induced in COVID patients that had been admitted to ICU vs. non-ICU COVID patients and Influenza patients, suggesting these surface proteins to be markers of disease severity.

It has previously been shown that CD9 is increased on circulating vesicles from COVID-19 patients when compared to healthy donors. Furthermore, the same study showed a pro-coagulant activity of these vesicles due to high levels of CD142 (tissue factor), as measured by flow cytometry. Therefore, it was hypothesized that these vesicles act as initiators of the coagulation cascade [12]. We have found slightly higher levels of CD9 on vesicles from Influenza patients compared to vesicles from COVID-19 patients. This observation was more pronounced when we limited our observation to CD9/CD41 double positive vesicles, yielding insight into a specific subset of sEV that was inaccessible with the EV array technique. CD41 suggested platelet origin of these EVs. Compared to CD9, CD142 was only weakly detected in our assay, so we do not find evidence of sEV pro-coagulant activity due to CD142. While the small sample size of influenza patients precludes a disease-specific conclusion, we show a significant global positive association between CD9 and the thrombocyte value. This observation corroborates our hypothesis of the thrombocytic origin of the CD9 positive vesicles we observe. It has been described before that Influenza virus activates platelets in a mouse model [15,16]. Furthermore, it has been hypothesized that platelets serve to carry Influenza virions to sites that are distant to the primary infection site [17]. It is therefore conceivable that the high load of sEVs with probable platelet origin that we observe in plasma samples of Influenza patients is a virulence feature of Influenza virus infection. Besides correlation with the thrombocyte value, there was also a tendency of CD9 to correlate with other clinical paramterers, such as body temperature, sodium value, and CRP, but not to a significant extent.

In addition to surface marker decoration, the cargo of vesicles has also been shown to be a function of infection. Analysis of bronchoalveolar lavage fluid (BALF) from patients with influenza-induced ARDS revealed an EV-associated miRNA profile linked to disease severity. Notably, among nine dysregulated miRNAs in BALF sEVs, miR-17-5p was highly upregulated in both patient samples and in exosomes from influenza-infected lung epithelial cells. Functionally, transfer of miR-17-5p via exosomes to naive lung cells downregulated the antiviral factor M×1 and enhanced viral replication, underscoring a potential biomarker and mechanistic role for this miRNA in severe influenza [18]. A 2021 cohort study (54 influenza patients: 25 A and 29 B) profiled serum exosomal microRNAs (miRNAs) and found distinctive changes during infection. Influenza B patients showed 76 miRNAs significantly altered versus healthy controls, while influenza A patients had 26 miRNAs changed. Several of these miRNAs (e.g. hsa-miR-326, miR-15b-5p, miR-150-5p) correlated strongly with inflammatory cytokine levels such as IL-6, IL-17A, and IL-1β [19].

Other EV markers that we have not addressed in detail include, for example, the ESCRT proteins [20] and a wide variety of lipids [21].

As patients present at different time-points after symptom onset, parameter kinetics might vary. Further, specific limitations of our study include the lack of a severity index to subgroup the patients into a more severe and less severe group. We have therefore used the ICU admission as a proxy for disease severity, which is limited to the EV surface array cohort. Also, the imbalanced dataset of the EV Array measurement, leaning strongly toward the COVID-19 samples, constitutes a limitation. Furthermore, the investigation of sEV surface markers by vesicle array did not yield clear individual markers with discriminatory potential between COVID-19 and Influenza samples. Only their aggregation into a group and additional testing on an Exoview platform with independent samples revealed CD9 as most potent discriminator found by all our approaches. Our study focuses on the differences in vesicle

surface marker decoration between COVID and Influenza infection and therefore does not have a healthy control group. We have addressed the comparison of sEV surface marker decoration in lung infection vs. healthy donors in a previous study [22].

While COVID-19 strain evolution would have been a very intriguing parameter to include into our models, the time of patient recruitment, which was before 23 March 2021, predates the onset of COVID-19 strain diversification.

In summary, we can show CD41/CD9 positive sEVs to be more abundant in Influenza infection vs. COVID-19 infection. Furthermore, CD9 abundance significantly associated positively with the thrombocyte value. Thrombocyte numbers are an important clinical parameter, as they indicate risk for vascular thrombosis [16]. Our finding might aid diagnostic work in the clinic to early on differentiate between COVID-19 and Influenza, which is crucial for adequate patient treatment. Additionally, differences in surface marker abundance between COVID-19 and Influenza disease might help elucidate the respective pathophysiology and even provide therapeutic disease-specific targets in the future.

As we do see differences in surface marker abundance, it is tempting to speculate that also the vesical cargo (protein and RNA) might be different between the two disease entities. In the future, we will elaborate on this question in order to substantiate our claim that sEVs from plasma are different between COVID-19 and Influenza disease.

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Members of the publishing CAPNETZ study network 2023

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WB and FKR performed the experiments, did the calculations, and wrote the manuscript.

RB and MJ performed and analysed the EV Array and proofread the manuscript.

ALJ and KL interpreted the ExoView analyses and proofread the manuscript.

ML, BW, LNS, and GR provided expertise on Influenza and COVID-19 disease and proofread the manuscript.

GB (for Members of the CAPNETZ study group) provided samples and clinical data, as well as project administration.

NB and BS acquired funding, supervised the project, and proofread the manuscript.

All authors read and approved the final manuscript.

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CRediT: **Wilhelm Bertrams:** Data curation, Formal analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing; **Fabienne K. Roessler:** Data curation, Methodology, Visualization, Writing – original draft, Writing – review & editing; **Rikke Bæk:** Investigation, Methodology, Writing – review & editing; **Anna Lena Jung:** Investigation, Methodology, Writing – review & editing; **Katrin Laakmann:** Investigation, Methodology, Writing – review & editing; **Malene Møller Jørgensen:** Investigation, Methodology, Writing – review & editing; **Mareike Lehmann:** Investigation, Writing – review & editing; **Barbara Weckler:** Investigation, Writing – review & editing; **Leon N. Schulte:** Investigation, Writing – review & editing; **Gernot Rohde:** Investigation, Writing – review & editing; **Nadav Bar:** Conceptualization, Funding acquisition, Resources, Software, Supervision, Validation, Writing – review & editing; **Grit Barten:** Project administration, Resources; **Bernd Schmeck:** Conceptualization, Funding acquisition, Investigation, Project administration, Resources, Supervision, Writing – review & editing.

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Availability of data and material

The EV Array dataset that supports the findings of this study is available on the figshare database under https://doi.org/10.6084/m9.figshare.28009130 [23]. Linked supplemental methods are available under https://doi.org/10.6084/m9.figshare.28574225.

Disclosure statement

No potential conflict of interest was reported by the author(s).

Consent

Signed informed consent was obtained from every single individual for prospective biobanking.

Ethics approval

A central IRB approval is available by the Ethics Committee of the Hannover Medical School; Project approval number: 301–2008.

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