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# Predictive value of baseline alpha defensin level in patients with stable coronary artery disease: A retrospective single center study

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ABSTRACT

*Background:* Inflammation plays a central role in atherogenesis. The major neutrophilic peptide alpha-defensin is a promising evolving risk factor for atherosclerosis. The aim of the present study was to examine the role of alpha-defensin in predicting future major adverse cardiovascular events (MACE) occurrence in fully revascularized patients with stable CAD under optimal medical therapy.

*Methods and results:* We retrospectively examined the prognostic value of baseline plasma alpha-defensin levels in predicting MACE occurrence in 174 fully revascularized patients for stable CAD between March 2016 and January 2017. Alpha-defensin levels were found 20 % higher among demised patients (10,859 pg/ml, IQR [6,920 to 23,320] vs. 9,020 pg/ml, IQR [5,540 to 16,180] pg/ml, P = 0.15). The absolute increase in mortality risk in patients with alpha-defensin levels greater than the median values was 72.5 % (P = 0.33). Log-rank analysis proved both recurrent PCI for de novo lesions (14.9 % and 2.3 %) and the composite of mortality and recurrent PCI for de novo lesions (27.6 % vs. 9.2 %) were significantly related to alpha-defensin values greater than the median (>9200 pg/ml).

*Conclusion:* Baseline plasma alpha-defensin is an independent predictor of mortality and recurrent PCI among patients with stable CAD. Alpha-defensin may evolve as a promising factor in cardiovascular risk assessment beyond traditional risk factors. Targeting alpha-defensin to ameliorate MACE occurrence should be addressed in future studies.

## 1. Introduction

Coronary artery disease (CAD) is a major cause of mortality and morbidity worldwide[1]. Despite optimal control of the traditional risk factors, there is a substantial residual unmet risk of cardiovascular events[2]; hence, enhancing risk stratification for mortality and cardiovascular events among CAD patients is prudent. The latter could be achieved by identifying new predictors associated with accelerated atherosclerosis phenotype. Inflammation has repeatedly been implicated in the pathogenesis of CAD. Participation of multiple inflammatory agents in different stages of atherosclerosis was previously described[3–10]. Moreover, several proofs for therapeutic anti-inflammatory interventions were previously introduced; statins' induced hsCRP reduction was associated with cardiovascular risk reduction[11–13]; canakinumab, a monoclonal antibody against interleukin-1β (The CANTOS study), reduced the incidence of major cardiovascular (CV) events without affecting LDL levels[14]; and chronic colchicine (COLCOT trial[15]) administration for CAD patients mitigated CV risk.

Among the inflammatory milieu, the major neutrophilic peptide, alpha defensin was identified as a possible linkage between inflammation and the development of atherosclerosis. Increased alpha-defensin expression was previously documented in patients with coronary artery disease and diabetes mellitus[16–18]. Pathological assessment disclosed high abundance of alpha-defensin in atherosclerotic human coronary plaques[19] and proved significant correlation between the deposition of alpha-defensins in skin and the severity of coronary atherosclerosis[20]. Mechanistically, alpha defensins inhibit the

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degradation of low-density lipoprotein (LDL) and Lipoprotein (a) by vascular cells[21], increase their binding and retention in extracellular matrix of endothelial cells[22,23], and inhibit tissue-type plasminogen activator-mediated fibrinolysis[24]. Moreover, increased plasma alpha-defensin levels are associated with acute myocardial infarction[25] and mortality in patients with peripheral vascular disease[26]. Using a transgenic mice model, our group was the first to prove alpha-defensin as a potential risk factor for both atherosclerosis and atherothrombosis [27,28].

It is noteworthy that alpha-defensin secretion is substantially suppressed using colchicine[27], which is considered a novel emerging treatment for secondary prevention of CVD (class IIb, A), particularly in patients with residual CV risk[29]. The current study was designed to test for plausible association between plasma alpha-defensin baseline levels and long-term outcomes in fully revascularized, stable CAD patients beyond the classical CAD risk factors.

## 2. Methods

### 2.1. Study population

The current retrospective observational study included patients from a previous study conducted by our group (HYMC 64.14). The previous study enrolled adult patients above18 years old, who were non-urgently catheterized with coronary intervention for stable CAD (stable angina, positive stress test, positive thallium scintigraphy, or staged procedure), and had available plasma test for baseline alpha-defensin level obtained before angioplasty between the years 2016–2017. All patients followed were status after single vessel, non-complicated PCI at the indexed hospitalization, and had completed any planned percutaneous revascularization procedures in the previous study (HYMC 64.14). Ostial, bifurcation, chronic total occlusion, heavily calcified/non-dilatable, left main or tortuous cases were excluded, and only patients with uneventful PCI were included; cases that were complicated with side branch occlusion, flow limiting dissection, or slow flow phenomenon were not included. The cumulative labeling (index procedure lesions $>$ 50 % plus past segments treated and/or $>$ 50 % stenosis) of single, double, or triple vessel CAD according to alpha defensin levels below and above the median were recorded including left ventricular ejection fraction. Patients were pharmacologically treated according to international guidelines that included the intensive use of statins, antiplatelets, and relevant-updated treatment for diabetes and hypertension. Patients were excluded if they had severe heart failure, left ventricular ejection fraction of less than 35 %, stroke within the previous 3 months, myocardial necrosis, previous coronary-bypass surgery, active/recent infection, chronic inflammatory disease, anemia $<$ 10 mg/dl, severe renal disease with a serum creatinine level that was greater than two times the upper limit of the normal range; severe hepatic disease, drug or alcohol abuse, malignancy, and current or planned long-term systemic glucocorticoid therapy. A total of 174 consecutive stable coronary patients were included. The medical history and traditional risk factors were reported; diabetes was considered HbA1c $>$ 6.5 % and/or use of glucose-lowering medication, hyperlipidemia was defined as a total cholesterol level above 200 mg/dl and/or the use of antihyperlipidemic drugs, hypertension was defined as systolic blood pressure $\geq$ 140 mm Hg and/or diastolic blood pressure $\geq$ 90 mm Hg and/or on antihypertensive medication, and smoking status was considered in patients with active cigarette smoking. The study was conducted in accordance with the Declaration of Helsinki and approved by the HYMC Institutional Review Board (protocol code is 79–10-HYMC). A written informed consent was waived due to the retrospective design of the study. Patients were retrospectively followed for a total of 36 months.

### 2.2. Laboratory measurements

Blood samples were previously obtained as part of research study HYMC 64.14 via the radial sheath before PCI. Total cholesterol, low-density lipoprotein (LDL), C-reactive protein (CRP), and glycosylated hemoglobin (HbA1c) were measured by routine in-house analyses. Samples for alpha-defensin test were stored after centrifugation as EDTA-plasma at $-80^{\circ}$ C until alpha-defensin levels were determined, within a period not exceeding 3 months. Plasma concentrations of alpha-defensin levels were measured using sandwich ELISA kits according to the manufacturers' protocols (HyCult Biotechnology, Kit#-HK317). Alpha-defensin concentrations were calculated from the standard curves. The lower limit of detection was 156 pg/mL.

### 2.3. Clinical outcomes and data collection

Data were collected including demographic, clinical, angiographic, laboratory, and procedural information. The primary efficacy end point was the composite of death from cardiovascular causes and recurrent PCI for de novo lesions at 36 months of follow-up. The secondary end points included the components of the primary efficacy end point, target lesion revascularization and stroke. In case of recurrent PCI, a blinded senior interventionist labeled the PCI as for target lesion revascularization or de novo lesion. None of the patients was lost to follow-up.

### 2.4. Statistical analysis

Descriptive statistics in terms of mean, standard deviation, and percentage were calculated to the whole parameters in the study. Normal distribution of the continues parameters were tested by kolmogorov smirnov test. As alpha-defensin parameter was not normally distributed we used Mann Whitney *U* test and Kruskal Wallis with adjustment for multiple comparisons for differences between groups. In addition, alpha-defensin values were divided into four quarters. The differences between those four gropes and several independent parameters were demonstrated by Pearson chi square and Kruskal Wallis. The association between the primary composite outcomes (of mortality and de novo lesion) and alpha defensin was assessed using Kaplan-Meier curves and log rank (mantal cox) test. Pearson correlation was used to test the relation between Alpha defensive and several independent parameters (Age, BMI, CRP, Hb1c%, Cholesterol, TG, HDL and LDL). Univariate analysis model was constructed to predict death, PCI, Unstable angina, and stroke by several independent parameters. $P < 0.05$ was considered as significant. SPSS program version 28 was used for all statistical analysis.

## 3. Results

### 3.1. Baseline characteristics of the study patients

The study cohort consisted of 174 patients (77 % males), with mean age of 62.4 years (SD $\pm$ 10.4). Among the cohort, 28 % had a history of hypertension, 36 % hyperlipidemia, 48 % diabetes, 39 % chronic ischemic heart disease (CIHD = previous PCI or/and myocardial infarction), and 3 % previous stroke (Table 1). At essence, the prescribed medications at discharge were guidelines guided: 96–98 % were discharged on aspirin, 94–97 % on clopidogrel, 55–58 % on a β-blocker, 38–40 % on an angiotensin-converting enzyme inhibitor (ACEI) or angiotensin receptor blocker (ARB), and 96–98 % on a statin agent.

Patients were divided into two groups according to the median level of plasma alpha defensin (9,200 pg/ml). Patients with above the median alpha-defensin levels had significantly higher incidence of $\geq$ 2 CAD (84 % vs. 76 %, $p < 0.05$), but were less often labeled as CIHD. All other clinical and procedural variables were equally distributed between the alpha-defensin groups.

The correlation between alpha-defensin, as continuous variable and other variables showed that lower HbA1c% was associated with higher levels of alpha-defensins ($R = -0.234$, $P = 0.003$). There was no significant relationship between alpha-defensin and other variables including

**Table 1**
Baseline patients' characteristics according to median alpha-defensin levels.

| | All group (n = 174) | Alpha-defensin < 9200 (pg/ml) (n = 87) | Alpha-defensin > 9200 (pg/ml) (n = 87) | p-value |
|---|---|---|---|---|
| Age | 62.4 ± 10.5 | 61.3 ± 11.1 | 63.1 ± 10 | 0.35 |
| Gender | | | | 0.37 |
| Male | 77 % | 80 % | 74 % | |
| Female | 23 % | 20 % | 26 % | |
| Hypertension | 28 % | 26 % | 29 % | 0.83 |
| Smoking | 18 % | 18 % | 18 % | 1 |
| Hyperlipidemia | 36 % | 33 % | 39 % | 0.53 |
| BMI | 28.5 ± 5.9 | 27.9 ± 5 | 29.1 ± 6 | 0.54 |
| Diabetes | 48 % | 47 % | 48 % | 1 |
| Nephropathy | 30 % | 26 % | 33 % | 0.41 |
| Neuropathy | 11 % | 6 % | 17 % | 0.03 |
| Retinopathy | 12 % | 10 % | 14 % | 0.64 |
| Death | 10 % | 7 % | 13 % | 0.33 |
| CIHD | 39 % | 47 % | 31 % | 0.08 |
| CVA | 3 % | 0 % | 6 % | 0.059 |
| CRP | 8.4 | 8.8 | 8 | 0.1 |
| HbA1c% | 6.8 | 7.3 | 6.4 | 0.04 |
| Cholesterol | 151 | 151.5 | 150.7 | 0.2 |
| LDL | 82.5 | 81.7 | 83.2 | 0.25 |
| Ejection Fraction% | 59.5 | 58 | 61 | 0.42 |
| 1 Vessel Disease | 19.5 % | 23.8 % | 15.9 % | 0.11 |
| 2 Vessel Disease | 39 % | 32.5 % | 46.4 % | 0.04 |
| 3 Vessel Disease | 41 % | 43.8 % | 37.7 % | 0.03 |

BMI- body mass index, CIHD- chronic ischemic heart disease, CRP- c-reactive protein, HbA1c- hemoglobin A1c, LDL- low density cholesterol.

age, BMI, CRP, and lipid profile. Likewise, most of the categorical variables tested showed non-significantly higher alpha-defensin levels if present (Table 2).

### 3.2. Long term outcomes

The incidence of major adverse cardiac events during follow-up according to median alpha-defensin level is summarized in Table 3. During a three-year follow-up period 17 patients (10 %) died from a CV event. The mortality rate was significantly higher among patients with above the median alpha-defensin levels, 12.6 % vs. 6.9 %, p = 0.02.

The unadjusted overall mortality risk increased by increasing alpha-defensin level, with an absolute increase of mortality of 83.3 % in patients with alpha-defensin level higher than the median cohort value (p = 0.31). Of note, the median time elapsed from discharge to mortality decreased with increasing alpha-defensin levels, 14.5 months, IQR [8.2 to 19.6] months vs. 21.3 months, IQR [18.8 to 24.7] months, with alpha-defensin levels above and below the median, p = 0.15, respectively.

The need for recurrent PCI at 3 years follow-up was 14 %, at a median of 16, IQR [12 to 25] months. The indications for recurrent PCI for all lesions were unstable angina (50 %), ST elevation myocardial infarction (21 %), and non-ST elevation myocardial infarction (29 %). The recurrent PCI rate was significantly higher in patients with above

**Table 2**
Alpha-defensin levels with and without available categorical variables.

| | Absent condition Alpha-defensin (pg/ml) | Present condition Alpha-defensin(pg/ml) | p-value |
|---|---|---|---|
| Hypertension | 9070 [5374–16402] | 9344 [6458–15354] | 0.55 |
| Smoking | 9200 [5437–16293] | 9193 [5388–15113] | 0.94 |
| Hyperlipidemia | 8340 [5182–16180] | 9581[6600–16367] | 0.11 |
| Gender | 8790 [5234–16202] | 9862 [6670–16345] | 0.21 |
| Diabetes | 8885 [5380–15580] | 9269 [6415–16600] | 0.34 |
| Nephropathy | 8790 [5437–15415] | 9729 [6148–18386 | 0.16 |
| Neuropathy | 8558 [5519–15795] | 12,169 [8303–21526] | 0.061 |
| Retinopathy | 9020 [5380–16225] | 9627 [7165–15922] | 0.43 |

**Table 3**
Patients' outcomes according to median alpha-defensin values. PCI- percutaneous coronary intervention.

| | All group (n = 174) | Alpha-defensin < 9200 (pg/ml) (n = 87) | Alpha-defensin > 9200 (pg/ml) (n = 87) | p-value |
|---|---|---|---|---|
| Death | 17 (10 %) | 6 (6.9 %) | 11 (12.6 %) | 0.02 |
| Recurrent PCI | 24 (14 %) | 9 (10.3 %) | 15 (17.2 %) | 0.04 |
| De novo lesion PCI | 15 (9 %) | 2 (2.3 %) | 13 (14.9 %) | 0.005 |
| Death + De novo PCI | 29 (18.3 %) | 8 (9.2 %) | 21 (24.1 %) | 0.002 |

the median alpha-defensin levels compared to others, 17.2 % vs. 10.3 %, p = 0.04.

Specifically, regarding PCI for de novo coronary artery lesions the indications for intervention were unstable angina (40 %), ST elevation myocardial infarction (20 %), and non-ST elevation myocardial infarction (40 %). We found significantly higher incidence in the group with alpha-defensin level > 9200 (pg/ml), 15 % vs. 2.3 %, p = 0.005. The events occurred at a median time of 20 months in both the higher and lower than median alpha groups. Of note, both smoking (46.7 % vs. 15.7 %) and hyperlipidemia (73.3 % vs. 32.7 %) were significantly correlated with recurrent PCI for de novo lesions, p = 0.008 and 0.003, respectively. Interestingly, the occurrence of target lesion revascularization was non-significantly higher with alpha-defensin levels below the median value, 5.7 % vs. 3.4 %, p = 0.51, respectively.

For the combined end point of death and recurrent PCI for de novo lesions, 29 cases (18.3 %) occurred during the study period; the majority (75 %) occurred at alpha-defensin levels above the median value, p = 0.002. The Kaplan–Meier survival analysis (Fig. 1) demonstrated a significant difference (p = 0.02) in event-free survival at 3 years in patients with below the median pre-procedural alpha-defensin compared to patients with above the median pre-procedural alpha-defensin levels. Of note, the median alpha-defensin values tended to be higher among patients with events than event-free patients (10,860 pg/ml, IQR [8,346 to 15,338] pg/ml vs 8451 pg/ml, IQR [5,540 to 16,180] pg/ml, p = 0.1), while the CRP levels were significantly higher among patients with events (6.3 mg/l, IQR [3.7 to 11.4] mg/l vs 4 mg/l, IQR [1.8 to 7.2] mg/l, p = 0.005).

## 4. Discussion

Cardiovascular disease (CVD) is a leading cause for mortality and morbidity worldwide[30]. The main CVD burden is ascribed to traditional, modifiable risk factors, including hypertension, physical inactivity, obesity, diabetes, smoking, and hypercholesterolemia. Yet, despite optimal management of these factors, a considerable proportion of patients remain at residual risk for recurrent major CV events[31]. This unmet need emphasizes the clinical importance of novel biomarkers and potential therapeutic targets for better management of patients in order to overcome residual cardiovascular risk[32]. The current article discusses the potential role of the major neutrophilic peptide alpha defensin in CV risk stratification.

Alpha-defensin showed robust association with both all-cause mortality in heart failure patients[33], and CAD severity in stable coronary disease patients[20,34]. Moreover, alpha-defensin was mechanistically linked with both atherosclerosis[27] and atherothrombosis[28]. A causative role of alpha-defensin in atherogenisis was repeatedly pointed, including induction of monocyte adhesion and transmigration, acceleration of foam cell formation, amplification of platelets' activation and aggregation[35], inhibition of tPA and uPA mediated fibrinolysis [24,36], induction of endothelial dysfunction[37,38], dysregulation of lipid metabolism including amplification of LDL internalization and deposition in vascular beds[23,32], and augmentation of platelet activation[39].

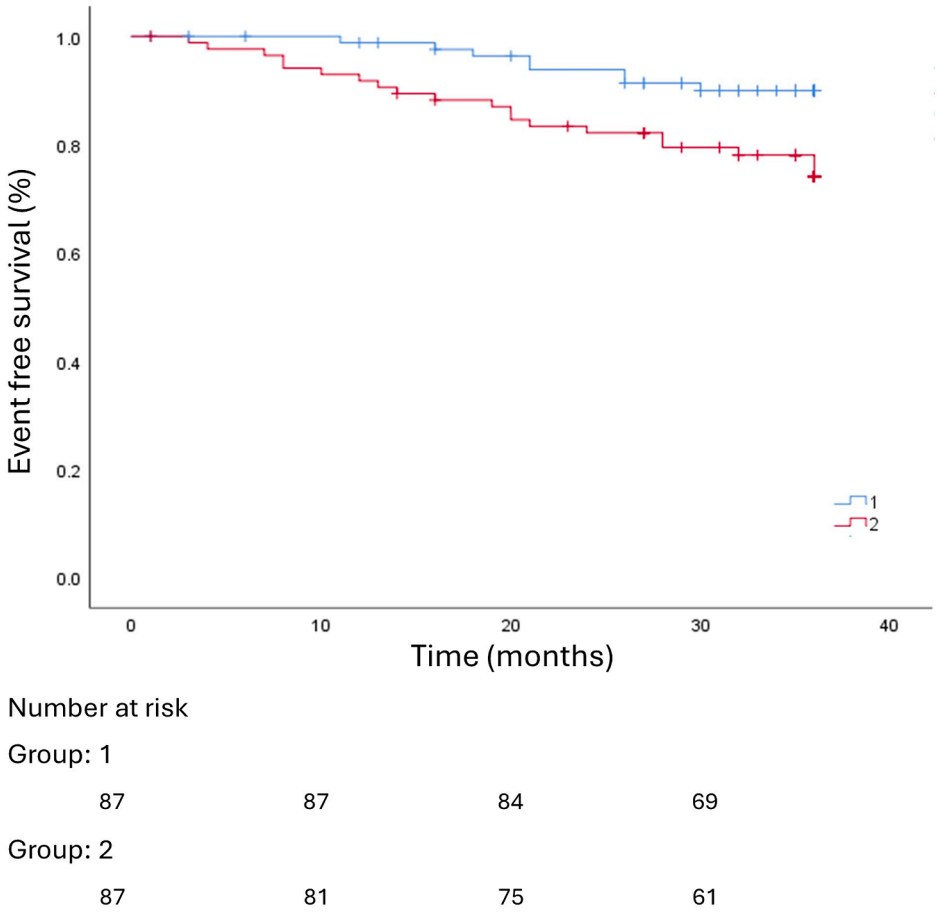


**Fig. 1. Kaplan-Meier curve analysis for patient stratification by the composite risk score.** Kaplan-Meier curves illustrating patient stratification by a composite risk score including mortality, and recurrent PCI for Denovo lesions, including the number at risk at each time interval. Patients with values below the median yielded the most favorable outcome (blue curve), whereas patients with values above the median demonstrated the worst survival rates (red curve). PCI indicates percutaneous coronary intervention. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

We found that in stable, fully revascularized CAD patients, receiving the standard of care treatment alpha-defensin $>$ 9200 (pg/ml) at baseline was significantly correlated with recurrent PCI, mortality, and the composite of recurrent PCI for de novo lesions and mortality, even after adjusting for baseline characteristics and treatments. Moreover, an elevated alpha-defensin was more often associated with shorter time to death from enrollment.

We also found higher occurrence of $\geq$ 2 CAD in patients with higher alpha defensin levels, which is in line with previous study showing higher incidence of multiple CAD with higher alpha defensin levels [20,34]. Intriguingly, alpha defensin related residual CAD risk was documented despite favorable HbA1c. This observation could be explained by a "glucose lowering effect" of alpha defensin described previously[27,40]. Regarding other established risk biomarkers, baseline CRP concentration did not predict recurrent PCI for de novo lesions, however, it was significantly associated with mortality. Nevertheless, CRP is mainly considered a risk marker lacking any solid data to justify specific or direct CRP targeting, whereas alpha-defensin exhibit multiple risk factor virtues and can potentially be suppressed and targeted by the anti-inflammatory agent, colchicine[29,41–43]. Colchicine stabilizes neutrophils including inhibition of alpha-defensin secretion. The association between alpha-defensin and both mortality and recurrent PCI for de novo lesions, together with the positive clinical effects of the alpha-defensin inhibitory agent, colchicine, call for future larger prospective research aiming to support the link between alpha defensin and major CV events, and possibly evaluating colchicine net effect in relation to baseline alpha defensin levels in stable CAD patients; such a research might support causality between alpha defensin and CVD risk.

## 5. Conclusions

Elevated alpha-defensin level is associated with higher risk of cardiovascular death, and recurrent PCI for de novo lesions in patients with stable CAD. Alpha-defensin levels might prove valuable is cardiovascular risk assessment in stable CAD patients, however, this supposition must be tested in future large-scale study.

### 5.1. *Strengths and limitations*

As mentioned, our study is the first study which examines long-term clinical outcomes of stable, fully revascularized CAD patients in relation to baseline plasma alpha defensin levels. Yet, this study has some limitations. First, the retrospective design of the current study negates any causative association between alpha defensin and CV outcomes. Second, the long-term adherence to medical therapy and/or the per-guidelines control of the different risk factors is beyond the scope of our study. Third, the normal range of alpha defensin found in plasma is in the nanomolar range, with a marked elevation during the acute inflammatory processes. Patients included were only stable patient, while excluding any potentially alpha defensin releasing triggers like acute coronary/vascular events, inflammatory processes, etc. As a results insights from this study are relevant only to stable coronary patients. Nevertheless, alpha defensin increase in acute coronary syndrome patients was also pointed previously[44]. Forth, the sample size is

relatively small to gain powerful results. Future larger prospective longitudinal research testing baseline alpha defensin level, including in different coronary disease population and the net effect of colchicine administration accordingly is mandatory to address alpha defensin clinical impact.

## Ethics approval

The study was conducted in accordance with the Declaration of Helsinki and approved by the HYMC Institutional Review Board (protocol code is 79–10-HYMC).

## 7. Patient consent statement

patients consent was waived due to the retrospective design of the study.

## CRediT authorship contribution statement

**Maanit Shapira:** Validation, Software, Investigation, Formal analysis. **Ariel Roguin:** Writing – review & editing, Investigation, Formal analysis. **Ibraheem Fayad:** Writing – review & editing, Supervision, Investigation, Formal analysis, Data curation. **Lina Medlij:** Writing – review & editing, Validation, Investigation, Formal analysis. **Aysha khateeb:** Writing – review & editing, Investigation, Data curation. **Dema Egbaria:** Writing – review & editing, Software, Investigation, Formal analysis. **Naama Amsalem:** Writing – review & editing, Project administration, Methodology, Formal analysis, Data curation. **Rami Abu Fanne:** Writing – review & editing, Writing – original draft, Validation, Supervision, Software, Project administration, Methodology, Investigation, Formal analysis, Conceptualization.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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