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# Network Motif Detection in the Network of Inflammatory Markers and Depression Symptoms among Patients with Stable Coronary Heart Disease: Insights from the Heart and Soul Study

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**Abstract:** Background. Network motif analysis is a technique used to explore recurrent and statistically significant subgraphs within a network. Applying a motif analysis to the complex network of inflammation and depression may yield nuanced insight into the specific interaction mechanisms between inflammatory markers and individual depression symptoms, which is our aim. Methods. This cross-sectional study is based on patients with stable coronary heart disease (CHD). A partial correlation network was initially constructed to link inflammatory markers, including C-reactive protein (CRP), Interleukin-6 (IL-6), tumor necrosis factor-α (TNF-α), monocyte chemoattractant protein-1 (MCP-1), individual depression symptoms, and covariates. A network-centric approach searched all possible non-isomorphic subgraph patterns of size $k = 4$ in the network. Results. Although CRP, IL-6, and TNF-α displayed an insignificant association with specific depression symptoms, the motif analysis revealed various subgraph patterns of interactions between depression symptoms associated with MCP-1. Generally, MCP-1 formed a closed loop with psychomotor problems and sleep disturbances, and this configuration was connected in various forms with other symptoms, particularly cognitive (e.g., feelings of worthlessness, concentration difficulty, and suicidal ideation) and neurovegetative/somatic (e.g., appetite changes and fatigue) symptoms. Moreover, MCP-1 was frequently associated with a closed-loop triangle comprising cognitive and neurovegetative/somatic symptoms but not with mood symptoms (e.g., loss of interest and feelings of sadness). Conclusions. The findings provide insight into how MCP-1 may be involved in the pathology of depression among patients with stable CHD in a more precise manner. This study also proposes future directions for research on depression.

**Keywords:** coronary heart disease; depression; inflammation; network motif



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## 1. Introduction

A body of literature has demonstrated that depression is linked to increases in inflammatory processes among patients with coronary heart disease (CHD) [1–3]. However, not all investigations have confirmed these relationships [4,5]. An explanation that recently received field-wide acceptance suggests that inflammation might be differentially related to specific depression symptoms or symptom subtypes and that any significant associations would be driven by these profiles [6,7]. This interpretation has prompted the study of psychopathology at the symptom level, which thus far has demonstrated that atypical forms of depression (e.g., somatic and neurovegetative symptoms) seem to drive associations of depression with inflammatory markers in CHD patients [8,9].

A notable aspect of these recent efforts is that researchers have increasingly shifted away from the "selective approach" of separately modeling the association of inflammatory markers with each specific depression symptom/subtype and instead increasingly embrace network analysis as a means to attain a more integral perspective on inflammation–symptom associations. Network analysis is a powerful tool that simultaneously estimates the pairwise conditional connections between all variables (referred to as "nodes") within a single mathematical framework [10]. In a network comprising inflammatory markers and depression symptoms, network analysis enables the complex interplay between variables to be revealed, identifying which specific symptom exhibits the most robust correlation with inflammatory markers while controlling for other variables (i.e., partial correlation). Studies have adopted this technique to explore dynamic inflammation–depression symptom associations involving various inflammation proteins such as C-reactive protein (CRP), Interleukin-6 (IL-6), and tumor necrosis factor-α (TNF-α) among diverse populations [11–16], which as of recently also include patients with stable CHD [9].

Network science enables us to comprehend complex systems, with a key aspect being the utilization of graph algorithms to identify and analyze unique substructures within these networks, known as 'subgraphs' or 'subnetworks.' These tools are essential for detecting patterns, such as tightly knit communities or pivotal nodes, that significantly influence the network's overall connectivity and functionality. This, in turn, provides deeper insights into the system's underlying properties and dynamics. There have been earlier attempts to detect these subgraphs within the network of inflammatory markers and psychiatric symptoms [17,18], primarily based on community detection algorithms (e.g., Walktrap algorithms, edge-betweenness approach, etc.), which define a subgraph as a set of nodes featuring more dense connections between its members than to the remainder of the network [19,20]. Essentially, community detection algorithms aim to identify naturally occurring groups within a network, regardless of their number or size, by dividing the network into clusters of vertices that share fewer connections with each other [20]. For instance, Santoso et al. [17] investigated the network structure of psychoneurological symptoms and stress/inflammation markers in newly diagnosed head and neck cancer patients and detected a distinct subgraph comprising a range of psychoneurological symptoms that were particularly associated with CRP and cortisol. Henneghan et al. [18] visualized a cytokine-symptom network (perceived stress, fatigue, loneliness, cognitive impairment, daytime sleepiness, sleep quality, and 13 cytokines) among breast cancer survivors and identified unique subgraphs within this network. From a network perspective, such a subgraph can pinpoint a group of nodes that may be readily influenced when a node included in the subgraph changes state [21]; for example, more densely connected variables may feature stronger mutual feedback.

To date, community detection algorithms typically offer a broader-level overview of densely connected nodes, without providing details of the structural properties or characteristics of the subgraph; however, it is probable that more intricate, higher-order connectivity patterns are present among the interconnected nodes of inflammation markers and symptoms, which may be highly informative. For uncovering these structural patterns, network motif analysis is an important tool [22]. In brief, a network motif is another concept describing local properties of a network and is defined as subgraphs or patterns of interconnections occurring in complex networks at numbers significantly higher than those in randomized networks, emphasizing statistical importance [23].

A network motif is selected based on its structural uniqueness, and the search involves counting all possible network configurations or connectivity patterns (refer to more details with Kim et al. [22]). The total number of non-isomorphic subgraphs or network motifs depends on the number of nodes being considered. For instance, for a structural motif size of $k = 4$, the method can detect a total of six non-isomorphic size-4 graph patterns from an undirected network, as shown in Figure 1, and recognize some patterns as network motifs after statistical analysis. Overall, the network motif analysis approach may provide a significantly more refined picture of meaningful subgraph structures in a given network than has previously been appreciated.

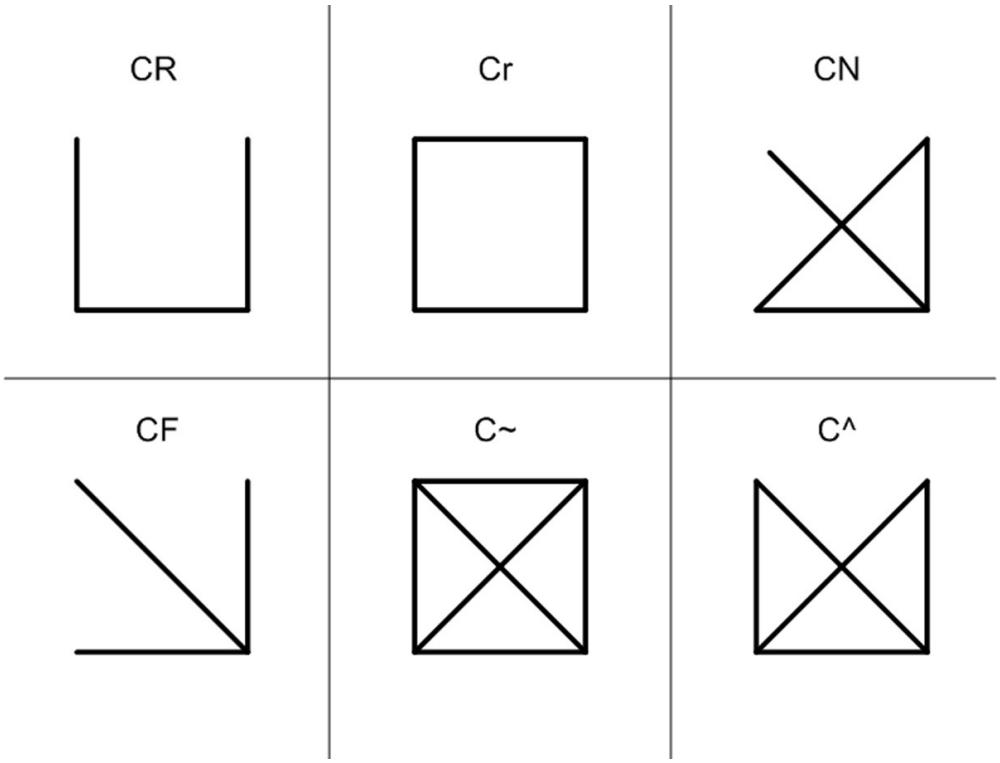



**Figure 1.** Shapes and labels for 4-node subgraphs in an undirected network. Note. The figure from Kim et al. [22]. There are six types for 4-node subgraph in an undirected network. CR, Cr, CN, CF, C~, and Cˆ are the canonical labels of each subgraph that determine whether the two graphs are isomorphic. If two graphs have the same canonical label, then they are isomorphic; otherwise, they are non-isomorphic. The canonical label is calculated by the Nauty program (https://pallini.di.uniroma1.it/).

Network motif analysis has predominantly found application in the field of systems biology, for instance in areas such as predicting essential proteins [24], identifying breast cancer-related genes [25], and determining protein interactions [26]. However, there are no theoretical barriers to applying network motif analysis to any type of networked data. It is a versatile statistical method that can be used to identify network motifs in complex networks across many fields of science, such as social [27] or semantic networks [28]. Without exception, all these networks can be represented as graphs, which include a wide variety of subgraphs. Applying motif analysis to the network of inflammation- and depression-related factors may yield nuanced insights into the specific interaction mechanisms between inflammatory markers and individual depression symptoms.

*Study Aims*

In summary, this exploratory investigation used network motif analysis to identify frequent and statistically unique subgraph patterns ("network motifs") in a network of inflammation and depression symptoms among patients with established CHD. As the first study to apply this approach to immunopsychiatry, we believe that our study can take the field a substantial step forward and ultimately help improve patient outcomes.

## 2. Materials and Methods

### *2.1. Dataset and Sample*

This study is a cross-sectional analysis of baseline data from the Heart and Soul Study. The Heart and Soul Study is a prospective cohort study originally designed to investigate how psychological disorders lead to CHD events in outpatients with stable CHD. The enrollment process and methods have been previously described in another study [29]. Briefly, patients were recruited from clinics at the San Francisco Veterans Affairs (VA)

Medical Center, the Palo Alto VA Health Care System, the University of California San Francisco Medical Center, and the San Francisco Community Health Network. Patients were considered eligible for study participation if they fulfilled at least one of the following criteria: a history of myocardial infarction (MI), angiographic evidence of at least 50% stenosis in one or more coronary vessels, previous evidence of exercise-induced ischemia using treadmill or nuclear testing, or a history of coronary revascularization. Exclusion criteria included a history of myocardial infarction in the past 6 months, self-perceived inability to walk for a distance of one block, or having plans to move out of the local area within 3 years. A baseline visit consisting of an in-person interview, the completion of a questionnaire, echocardiogram recordings of baseline and stress values from an exercise treadmill test, and a 12 h fasting blood draw was completed by all participants.

From September 2000 to December 2002, a total of 1024 patients enrolled in the study. Out of this initial sample, we removed participants with incomplete data (listwise deletion). Following this step, the study retained a total of 967 participants (81.9% men) who were used as the primary sources of data for analysis.

### *2.2. Measures*

#### 2.2.1. Inflammation Markers and Covariates

Markers of inflammation included CRP, IL-6, TNF- $\alpha$, and monocyte chemoattractant protein-1 (MCP-1). Participants were instructed to fast for 12 h (except for medication consumable with water), avoid consuming aspirin for 1 week, and avoid smoking for 5 h before their appointment. Venous blood samples were obtained, and plasma and serum samples were stored at $-70$ °C. The laboratory technicians performing the inflammatory marker assays were blinded to the results of the depression interview. High-sensitivity CRP levels were determined using the Roche Integra or Beckman Extended Range assays. The results obtained from these two assays exhibited a highly significant correlation. ($r = 0.99$). To measure IL-6, TNF-$\alpha$, and MCP-1 levels, the Human Serum Adipokine Panel B LINCOplex Kit (Linco Research, Inc. St. Charles, MO, USA, subsequently acquired by Millipore), was utilized. In order to account for potential confounding effects, age and body mass index (BMI) were included as covariates in the analyses.

#### 2.2.2. Depression Symptoms

The nine-item self-reported patient health questionnaire (PHQ-9) was used to measure the following nine individual depressive symptoms listed in the Diagnostic and Statistical Manual of Mental Disorders-Fourth Edition [30]: (1) psychomotor/cognitive symptoms, including psychomotor problems, feelings of worthlessness, concentration difficulty, and suicidal ideation; (2) mood symptoms, such as loss of interest and feelings of sadness; and (3) neurovegetative/somatic symptoms, including appetite changes, fatigue, and sleep disturbance [31]. The questionnaire evaluates symptoms over the previous 2 weeks rated on a scale of 0 = not at all, 1 = several days, 2 = more than half of the days, or 3 = nearly every day.

### *2.3. Statistical Analysis*

#### 2.3.1. Correlation Network Construction

In the current study, a partial correlation was employed to construct networks where the coefficient was calculated based on the correlation between two variables, considering the correlation with the remaining set of variables. Prior to the network construction, the data were organized in a comma separated values (CSV) file in a tabular form, with columns representing the variables (inflammatory markers, individual depression symptoms, and covariates) and rows representing the patient records. This CSV file was uploaded to the Java-based CorrelationCalculator software (v.1.0.1; http://metscape.ncibi.org/calculator.html) accessed on 23 August 2023 for correlation analysis. As most inflammatory biomarkers exhibit an abnormal distribution and are highly skewed, the data were normalized using the "autoscale data" option. The correlation analysis was performed using a debiased sparse partial correlation (DSPC) algorithm. In the final step, the CorrelationCalculator gen-

erated a CSV file with the correlated variables in the first two columns, partial correlation coefficients in the third, unadjusted *p*-values in the fourth, and adjusted *p*-values in the fifth. We selectively included only significant correlations, setting a threshold of adjusted *p*-value < 0.05. Variables that met this significance criterion (identified in the first and second columns) were extracted and saved in a text (.txt) file. This file was subsequently imported into Cytoscape software (v.3.7.0; cytoscape.org) to construct the (unweighted) network, as illustrated in Figure 2. A correlation network was constructed as an undirected graph due to the cross-sectional nature of the study design. Each network "node" represents an inflammatory marker, individual depression symptom, or covariates, and each "edge" represents the partial correlation coefficient between two nodes.

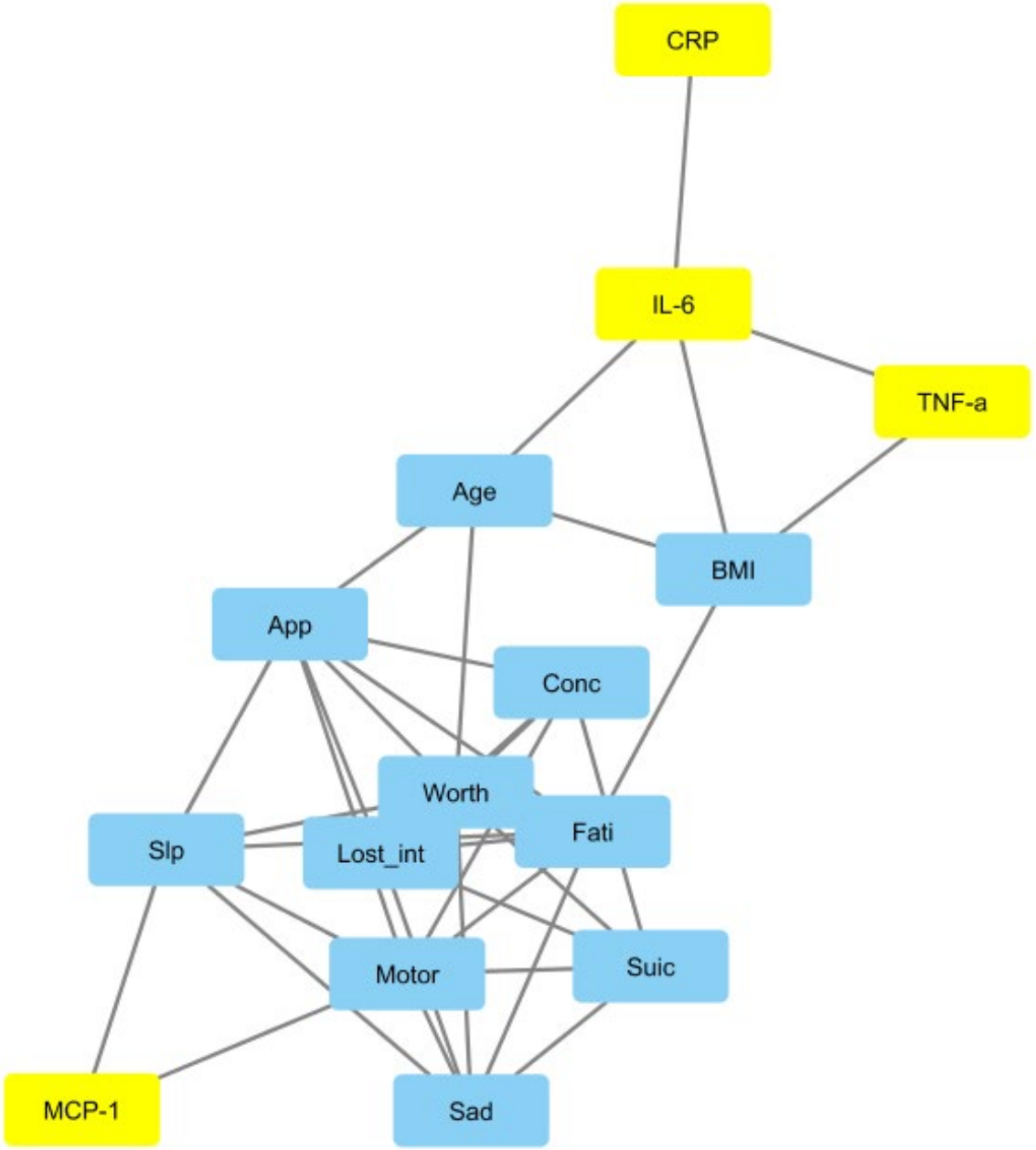



**Figure 2.** Correlation network. Note. Lost_int, loss of interest; App, appetite changes; Conc, concentration difficulty; Suic, suicidal ideation; Fati, fatigue; Worth, feelings of worthlessness; Motor, psychomotor problems; Sad, feelings of sadness; Slp, sleep disturbance. The Inflammatory markers are color-coded in yellow. The graph was depicted as an unweighted graph; the correlation values for each can be found in the Table S1.

Table S1 displays the significant partial correlations ($r$) with adjusted $p$-values less than 0.05, while Figure 2 visually represents the constructed correlation network. CRP, IL-6, and TNF-α did not correlate directly with individual depression symptoms. However, MCP-1 displayed significant correlations with "sleep disturbance" ($r$ = 0.107, adjusted $p$ = 0.022) and "psychomotor problems" ($r$ = 0.099, adjusted $p$ = 0.031). Interestingly, "psychomotor problems" displayed the most connections to other individual depression items and inflammatory markers within the network.

#### 2.3.2. Network Motif Analysis

The web program NemoSuite (Network Motif Analysis in a Suite; https://bioresearch.css.uwb.edu/biores/NemoSuite/) accessed on 23 August 2023 developed by Kim et al. [32] was applied to detect and analyze network motifs. Although network motifs may provide deep insight into the network's functional abilities, their detection is computationally challenging and not well supported by existing systems. NemoSuite is an easily accessible and highly usable tool that is available in a web-interactive and graphical user interface format [32]. It offers great efficiency that current network motif detection software programs lack, such as various input and output options, enabling users to find efficient solutions for motif detection. In addition, this is the first tool that provides both network and motif-centric approaches using two programs, **Nemo** and **NemoMapPy.** The **Nemo** program implements a network-centric approach using NemoLib, while the **NemoMapPy** program implements the NemoMap algorithm, one of the motif-centric methods. A network-centric approach, which was employed in our study, is particularly useful if the user aims to detect which patterns are network motifs. This approach searches for all possible non-isomorphic subgraph patterns within a specified size range (typically three to eight) in the target network, whereas the motif-centric method counts the occurrence of a predetermined query graph pattern in the original network [33]. The frequency of each pattern is compared in a random pool to ascertain whether the frequency is statistically high, eventually yielding statically verified frequent patterns (i.e., network motifs). The uniqueness of each pattern is determined based on the $p$-value or $z$-score. Generally, a subgraph with a $p$-value of less than 0.01 or a $z$-score higher than 2.0 is considered a network motif.

A network-centric approach requires an input network, directness, a motif size of $k$, and the number of random graphs to generate. We used the text (.txt) file created in the initial step (this time, without the column names) as an input network and uploaded it to the **Nemo** program. An "undirected graph" was chosen, given the cross-sectional nature of the study design. The motif size ($k$) was set to four, considering the computational efficiency, interpretability, and statistical robustness, and the random graph size was set to 10 [32]. A network-centric approach offers three output options—NemoCount, NemoProfile, and NemoCollection—whose formats were introduced by Kim and Haukap [34]. The NemoCount option returns the patterns of each detected subgraph, with its frequency in the input network and average frequency in random graphs. Finally, it demonstrates its statistical results so the user can identify which pattern is a network motif. This output is provided for all options by default. However, the NemoProfile, and NemoCollection options provide additional files. The NemoProfile option generates a *SubgraphProfile* file that comprises a two-dimensional matrix with $i$ rows and $j$ columns, where n represents the total number of nodes present in the input graph, and m corresponds to the number of subgraph patterns generated in the target network. The value at ($i$, $j$) signifies the number of occurrences of the $i^{\text{th}}$ node in the $j^{th}$ subgraph. From the *SubgraphProfile*, users can select the columns that correspond to network motifs, which can be used for the *NemoProfile*. The NemoCollection option offers a file, *SubgraphCollection*, which lists all instances of each subgraph pattern. From this file, users can easily generate a *NemoCollection* by collecting only the instances of network motifs.

## 3. Results

Table 1 presents the NemoCount results. We identified a total of 99 instances of "CR" size-4 subgraphs (48.8%), 8 instances of "Cr" size 4-subgraphs (3.9%), 53 instances of "CN" size-4 subgraphs (26.1%), 20 instances of "CF" size-4 subgraphs (9.9%), one instance of "C~" size-4 subgraphs (0.5%), and 22 instances of "Cˆ" size-4 subgraphs (10.8%). The "CN," "C~," and "Cˆ" patterns were identified as network motifs through statistical analysis, indicated by a *p*-value of less than 0.01 or a *z*-score greater than 2.0. Table 2 presents the NemoProfile matrix (A), where A*ij* represents the frequency of node *i*'s involvement in subgraph *j*; notably, MCP-1 is the most recurrently involved inflammatory marker in these motifs ("CN," "C~," and "Cˆ" patterns).

**Table 1.** NemoCount results.

| Label | Pattern | Frequency [Original] | Mean Frequency in the Randomized Networks | *z*-Score | *p*-Value |
|---|---|---|---|---|---|
| CR |  | 48.8% | 61.7% | −3.54 | 1.000 |
| Cr |  | 3.9% | 4.1% | −0.07 | 0.400 |
| **CN** |  | **26.1%** | **13.9%** | **3.87** | **0.000** |
| CF |  | 9.9% | 18.6% | −2.68 | 1.000 |
| **C~** |  | **0.5%** | **0.1%** | **2.21** | **0.100** |
| **Cˆ** |  | **10.8%** | **1.8%** | **7.33** | **0.000** |

Note. "CN," "C~," and "Cˆ" patterns, which are labeled in bold, were determined as network motifs.

**Table 2.** NemoProfile results.

| Node | CR | Cr | CN | CF | C~ | Cˆ |
|---|---|---|---|---|---|---|
| |  |  |  |  |  |  |
| Psychomotor problems | 28 | 6 | **22** | 8 | **1** | **11** |
| Feelings of worthlessness | 37 | 4 | **17** | 6 | **0** | **10** |
| Concentration difficulty | 25 | 2 | **16** | 6 | **0** | **14** |
| Suicidal ideation | 25 | 5 | **14** | 6 | **0** | **6** |
| Loss of interest | 41 | 4 | **14** | 10 | **0** | **8** |
| Feelings of sadness | 15 | 0 | **3** | 3 | **0** | **0** |
| Appetite changes | 34 | 4 | **33** | 7 | **1** | **17** |
| Fatigue | 41 | 3 | **25** | 9 | **1** | **6** |
| Sleep disturbance | 31 | 2 | **21** | 6 | **1** | **8** |
| CRP | 3 | 0 | **2** | 1 | **0** | **0** |
| IL-6 | 17 | 0 | **7** | 1 | **0** | **1** |
| TNF-α | 8 | 0 | **2** | 2 | **0** | **1** |
| MCP-1 | 17 | 0 | **8** | 4 | **0** | **2** |
| Age | 38 | 1 | **17** | 8 | **0** | **3** |
| BMI | 32 | 1 | **11** | 3 | **0** | **1** |

Note. "CN," "C~," and "Cˆ" patterns, which are labeled in bold, were determined as network motifs.

Table S2 provides the NemoCollection results. Among the "CN" patterns, the one that illustrates the connections between inflammatory markers and depression symptoms includes the following: psychomotor problems, suicidal ideation, concentration difficulty, and MCP-1; psychomotor problems, suicidal ideation, MCP-1, and sleep disturbance; psychomotor problems, fatigue, appetite changes, and MCP-1; psychomotor problems, appetite changes, concentration difficulty, and MCP-1; psychomotor problems, concentration difficulty, MCP-1, and sleep disturbance; psychomotor problems, MCP-1, sleep disturbance, and feelings of worthlessness; BMI, fatigue, IL-6, and age; BMI, fatigue, IL-6, and TNF-α; BMI, IL-6, age, and appetite changes; BMI, IL-6, age, and feelings of worthlessness; fatigue, appetite changes, sleep disturbance, MCP-1; appetite changes, feelings of worthlessness, sleep disturbance, and MCP-1; and appetite changes, feelings of worthlessness, age, and IL-6]. The "C~" pattern comprises only depression symptoms. In the case of the "C^" patterns, the following motifs exhibit connections between depression symptoms and inflammatory markers: psychomotor problems, fatigue, MCP-1, and disturbance; and psychomotor problems, appetite changes, MCP-1, and sleep disturbance.

It is important to note that the NemoSuite program does not provide a graphical representation for each motif. Therefore, each motif must be inferred by referencing the constructed correlation network (Figure 2) based on the NemoCollection results. Thus, the manually drawn graphical presentation of these motifs is shown in Table 3. Generally, MCP-1 formed a closed loop with psychomotor problems and sleep disturbances, and this configuration was connected in various forms with other symptoms, particularly cognitive (e.g., feelings of worthlessness, concentration difficulty, and suicidal ideation) and neurovegetative/somatic (e.g., appetite changes and fatigue) symptoms.

**Table 3.** Examples of "CN", "C~", and "C^" pattern inferred from the constructed correlation network.

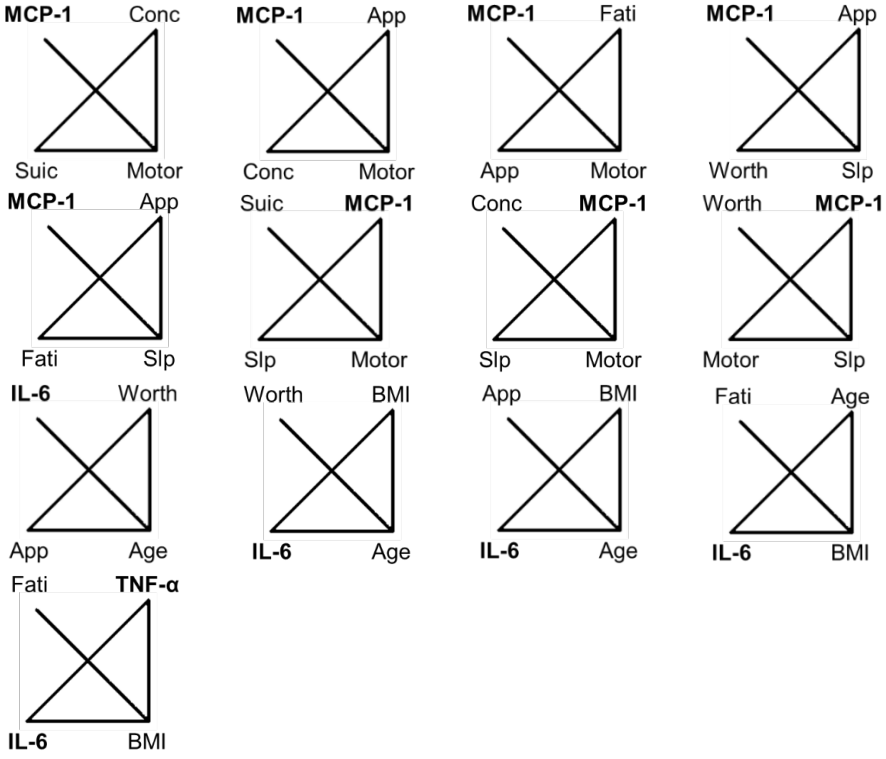
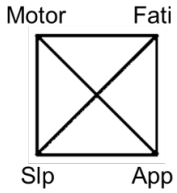
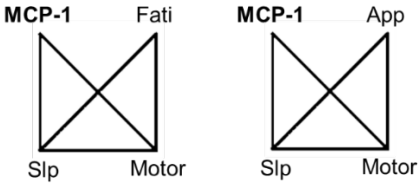
| Label | Pattern | Examples |
|---|---|---|
| CN |  |   |

**Table 3.** *Cont.*

| Label | Pattern | Examples |
| --- | --- | --- |
| C~ |  |   |
| Cˆ |  |   |

Note. App, appetite changes; Conc, concentration difficulty; Suic, suicidal ideation; Fati, fatigue; Worth, feelings of worthlessness; Motor, psychomotor problems; Slp, sleep disturbance. Inflammatory markers were written in bold. The NemoSuite program lacks the capability to generate graphical representations for all motifs. Consequently, each motif was manually deduced from the established correlation network illustrated in Figure 2.

## 4. Discussion

Using the web program NemoSuite, we detected motifs in a network of inflammatory markers and individual depression symptoms in patients with established CHD. Three non-isomorphic subgraphs of size 4, represented by the "CN," "C~," and "Cˆ" patterns, were identified as network motifs through statistical analysis, with criteria such as *p*-values or *z*-scores. Notably, MCP-1 appeared most frequently in the network motifs among inflammatory markers. After visualizing all instances of individual motifs that illustrate connections between inflammatory markers and depression symptoms, we obtained encouraging results regarding the distinct pattern each motif reveals.

MCP-1 is a chemokine released by activated microglia and potentially plays a crucial role in monocyte trafficking to the brain, which could lead to excitotoxic neuronal injury that induces depression [35]. Earlier studies have reported a positive correlation between MCP-1 and the severity of depression symptoms [36], including studies involving patients with type 2 diabetes [37]. Elevated MCP-1 levels in major depressive disorders were confirmed through a meta-analysis [38] in a recent study [39] and a study involving patients with congestive heart failure [40]. Unlike previous research, we recognized specific subgraphs or patterns of symptoms of depression using a motif-inspired paradigm in which MCP-1 may confer risk in patients with stable CHD.

The preliminary results of this study revealed that MCP-1 displays significant and direct correlations with psychomotor problems and sleep disturbances. Psychomotor problems (especially psychomotor slowing) are a cardinal symptom of major depressive disorders. Inflammation exerts specific effects on the basal ganglia regions that mediate inflammation-associated decreases in motivation and motor activity [41]. These effects may be partially related to the impact of inflammation on dopamine in key basal ganglia regions, including the ventral striatum [41]. To the best of our knowledge, only studies by Goldsmith et al. [42,43] have demonstrated that MCP-1 exhibits an association with decreased psychomotor speed. Furthermore, no study has demonstrated that MCP-1 is related to specific depression symptoms, such as sleep disturbances, in patients with stable CHD. Kazmi et al. [44] recently reported findings that demonstrate significant associations between MCP-1 and sleep quality. Additionally, a recent meta-analysis highlighted the relationship between higher levels of MCP-1 and the risk of obstructive sleep apnea [45].

Notably, the motif analysis results offer a number of intriguing insights into patterns of interactions between depression symptoms associated with MCP-1. In several individual motif instances, MCP-1 formed a closed loop with psychomotor problems and sleep disturbances. Specifically, fatigue and appetite changes, commonly referred to as "sickness behaviors" in

animal models of depressive illness, were associated with this closed-loop structure, forming a Cˆ pattern, suggesting a potential feedback or regulatory mechanism within this structure. Although the network in this study is treated as undirected, thereby omitting crucial directional information among specific symptoms in the Cˆ pattern, the results offer an opportunity for a more detailed understanding of MCP-1's critical role in depression pathology.

Moreover, MCP-1 was frequently associated with a closed-loop triangle comprising cognitive and neurovegetative/somatic symptoms. For instance, in one case with a CN pattern, MCP-1 was associated with a closed-loop triangle comprising psychomotor problems, concentration difficulty, and suicidal ideation with a direct link to psychomotor problems. In other instances, such as with the CN pattern, concentration difficulty and suicidal ideation were indirectly linked to MCP-1 via psychomotor problems. While no study has reported any association between MCP-1 and concentration difficulty in patients with CHD, MCP-1 has been linked to cognitive impairments in diverse clinical populations, including first-episode psychosis [46], cognitive deficits associated with HIV [47], mild cognitive impairment, early stages of Alzheimer's disease [48,49], and neurocognitive deficits after carotid endarterectomy [50]. Furthermore, MCP-1 exhibited increased expression in the brains of suicide victims [51]. Although it remains challenging to distinguish whether each component of the network motif acts as a moderator, mediator, or confounder, given the nature of the network analysis approach, these observations may support the results of previous studies.

In all individual motif instances, MCP-1 was not associated with mood symptoms (e.g., loss of interest and feelings of sadness; "pure depression"). The underlying etiology may vary across depression symptom constructs [52]. Given the results, MCP-1 appears to be more involved in cognitive and neurovegetative/somatic symptoms than in mood symptoms. The observed variations in specific depression symptoms related to MCP-1 in our study highlight the complex nature of depression, advocating for an approach that transcends simplistic aggregate scoring. However, it is important to recognize that empirical studies on MCP-1's role in depression have produced mixed outcomes, including findings of decreased MCP-1 levels in individuals with major depressive disorder [39,53–55]. These inconsistencies underscore the intricate relationship between inflammation markers and depression, emphasizing the need for further research in this domain.

As observed in past literature [56–58], increased levels of IL-6 were related to age and BMI in the sample. However, CRP, IL-6, and TNF-$\alpha$ did not indicate any direct association with specific depression symptoms, nor were they implicated in any motif instances. Previous network studies have demonstrated that several inflammation–depression (sum score) and inflammation–symptom relationships involving CRP, IL-6, and TNF-$\alpha$ are often attenuated by controlling for confounders, such as age and BMI [11–13,15,16]. A recent study that utilized various network analyses to explore how CRP is uniquely related to specific depression symptoms in patients with stable CHD [9] found that the association between CRP levels and depression symptoms weakened after adjusting for factors like age and BMI. Expressly, BMI is acknowledged as a significant confounding factor in the correlation between inflammation and depression [59], with adipose tissue acting as a proactive endocrine organ and producing a multitude of proinflammatory cytokines [60]. Furthermore, CRP cannot cross the blood–brain barrier and directly affect emotion-regulating areas in the brain [61].

Lastly, we underscore the distinctive application of the NemoProfile as a method of feature selection. As illustrated in Table 2, the NemoProfile saves the set of subgraph instances as structural matrices so that the frequency of each node's involvement in each subgraph pattern is recorded, while most motif-finding algorithms provide only the frequency and statistical significance of each pattern [62]. In addition, nodes frequently involved in network motifs may be more critical. If a node is involved in several distinct types of network motifs, it may potentially have multifunctional roles [63]. For example, Wang and Kim [64] found that NemoProfile can be used as a data feature to detect essential genes. By taking advantage of the NemoProfile approach, future research may replicate this study with a broader array of inflammatory markers and investigate the critical inflammatory agents that most frequently appear in the network motifs, which may be the essential feature in explaining depression.

### *4.1. Limitations*

As mentioned in the discussion, the first and primary limitation of this study is the cross-sectional design, in which a causative link between variables in network motifs cannot be directly inferred. While our results may offer fundamental insights into the inflammatory processes underlying depression, it is important to note that for network analysis to effectively inform intervention strategies, treatment decisions, and diagnoses, longitudinal designs are necessary [65]. These designs would allow us to model changes in nodes (e.g., symptoms) over time [65]. Second, the generalizability of the findings is limited, as patient recruitment was from only one site, which predominantly comprised males. Third, there is a risk of residual confounding from factors associated with the levels of inflammatory markers and depression, including medical health status, medication use, alcohol dependence, and cigarette use, which may serve as significant sources of bias and should be addressed in future studies. Fourth, although the motif size ($k$) was set to four in this study, considering the computational efficiency, interpretability, and statistical robustness, the findings may have been different if we set out to measure the motif distribution of sizes greater than four. Thus, we regard this study as exploratory. Fifth, the process of identifying network motifs is based on an unweighted network, which presents a limitation in interpreting each network motif instance because it does not consider the edge weight of each association. Therefore, caution is required when interpreting the results. Finally, direct comparisons of our findings with those from earlier network analysis studies on inflammation and depression [9,11–13,15,16] may not be straightforward. Variations in partial correlation calculations, sample sizes, node selection, and covariate types may make these comparisons challenging. Additionally, while prior research has largely centered on CRP, our study represents the initial foray into using network motif analysis to explore the association between inflammation and depression, incorporating a wider array of inflammatory markers. Consequently, our findings should be considered highly exploratory.

### *4.2. Future Directions*

The NemoSuite program provides the results as text output without a means of visual analysis (i.e., a visual representation of the motif structure). As Kim et al. (2020) noted, future work should add more utility programs, such as motif visualization and graph file conversion. In addition, the conclusions regarding the direction of the association were limited by the PHQ-9, as some items were double barreled. For instance, changes in psychomotor functioning (i.e., psychomotor slowing or agitation) were represented by the same criterion for depression. Accordingly, leveraging more fine-grained information on depression symptoms (through other scales) is an essential step in furthering these research efforts.

## 5. Conclusions

Research on depression still has much to uncover, and we believe that a network motif analysis is a promising methodology that could demystify its etiology. The motivation for this exploratory investigation is to promote this motif-inspired paradigm to uncover the mechanisms of inflammation-depression symptoms in patients with established CHD. If this study were replicated with a more diverse range of inflammatory markers and more fine-grained depression items, the findings could inform more effective anti-inflammatory strategies for more precisely managing depression.

**Supplementary Materials:** The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/psycholint6020027/s1, Table S1: Significant partial correlations (r) with adjusted $p$-values less than 0.05; Table S2: NemoCollection results.

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**Informed Consent Statement:** Informed consent was obtained from all subjects involved in the study.

**Data Availability Statement:** The datasets used and analyzed during the current study are available by submitting a proposal request to the Heart and Soul Study team (https://heartandsoulstudy.ucsf.edu/).

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