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Symptomatology following loss and trauma: Latent class and network analyses of prolonged grief disorder, posttraumatic stress disorder, and depression in a treatment-seeking trauma-exposed sample

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Background: Although bereavement is likely a common stressor among patients referred to a psychotrauma clinic, no study has yet examined the co-occurrence and relationships between symptoms of prolonged grief disorder (PGD), posttraumatic stress disorder (PTSD), and major depressive disorder symptoms in this population.

Method: In a sample of patients seeking treatment following psychological trauma ($n = 458$), we used latent class analysis to identify classes of patients sharing the same profile of PGD, PTSD, and depression symptoms. We then used network analysis to investigate the relationships among these symptoms and with loss-related variables.

Results: Most participants (65%) were members of a class that exhibited elevated endorsement of PGD symptoms. PGD, PTSD, and depression symptoms hung together as highly overlapping but distinguishable communities of symptoms. Symptoms related to social isolation and diminished sense of self bridged these communities. Violent loss was associated with more difficulty accepting the loss. The loss of close kin was most strongly associated with difficulty moving on in life.

Conclusions: PGD symptoms are common in trauma-exposed bereaved adults and closely associated with symptoms of PTSD and depression, illustrating the importance of assessing bereavement and PGD symptoms in those seeking treatment following trauma.

KEYWORDS
bereavement, depression, latent class analysis, network analysis, posttraumatic stress disorder, prolonged grief disorder, trauma

1 | INTRODUCTION

Nearly half of all patients receiving treatment for posttraumatic stress disorder (PTSD) fail to recover (Bradley, Greene, Russ, Dutra, & Westen, 2005). In groups that have experienced multiple traumas, recovery rates are even lower (Green et al., 2000; Nosè et al., 2017; Steenkamp, Litz, Hoge, & Marmar, 2015). Although PTSD is the disorder most commonly associated with trauma (de Vries & Olff, 2009; Foa, Stein, & McFarlane, 2006), trauma-exposed patients frequently present with additional co-occurring disorders (Brady, Killeen,

Brewerton, & Lucerini, 2000; Foa et al., 2006). To improve the efficacy of treatments for trauma-related psychopathology, it is critical to assess the full breadth of the complex clinical picture presented by these patients.

Bereavement is a potent stressor frequently experienced by those exposed to war, violence, disaster, accidents, and other traumatic events. It is a commonly reported index event among those with PTSD (Breslau, Chilcoat, Kessler, & Davis, 1999; Kessler et al., 1999), frequently precipitates depression (Cole & Dendukuri, 2003), and confers risk for many other mental disorders (Keyes et al., 2014;

Simon et al., 2007). Recently, researchers have also examined a bereavement-specific syndrome characterized by prolonged and impairing grief. This syndrome, which we will refer to as prolonged grief disorder (PGD), is listed as a diagnosis in need of further study in the 5th edition of the Diagnostic and Statistical Manual (American Psychiatric Association, 2013; under the name Persistent Complex Bereavement Disorder) and included in the 11th edition of the International Classification of Diseases (Maercker et al., 2013; Prigerson et al., 2009). An estimated 10% of bereaved adults confronted with natural loss will develop PGD (Lundorff, Holmgren, Zachariae, Farver-Vestergaard, & O'Connor, 2017) and there is some evidence that those experiencing violent loss are especially vulnerable to PGD as well as PTSD and depression (Djelantik, Smid, Kleber, & Boelen, 2017b; Kristensen, Weisaeth, & Heir, 2012; van Denderen, de Keijser, Kleen, & Boelen, 2015). Among bereaved adults, PGD is more strongly associated with elevated impairment than PTSD or depression (Boelen & Prigerson, 2007; Silverman et al., 2000). Consequently, failure to assess PGD may neglect an important part of the clinical picture presented by these patients. This is especially important because adequate treatments for depression, PGD, and PTSD differ (Boelen, 2006; Zisook & Shear, 2009) and there is evidence that treatments designed for depression are less effective in reducing PGD than are treatments that specifically target PGD (Shear et al., 2014).

In this study, we investigated whether bereavement and PGD are an important part of the clinical picture presented by individuals seeking treatment for trauma-related distress by examining rates of bereavement and symptoms from three syndromes that commonly accompany bereavement: PGD, PTSD, and depression. We focused our examination at the level of the symptom and investigated three primary aims.

First, we used latent class analysis (LCA), a person-centered analytic approach, to identify subgroups of patients sharing the same symptoms. In a prior study investigating PGD, PTSD, and depression symptoms in a bereaved community sample, three classes were identified: a resilient class with minimal symptoms, a class endorsing only PGD symptoms, and a class endorsing both PGD and PTSD symptoms (Djelantik et al., 2017b). We were principally interested in whether we would again observe classes characterized by elevated PGD symptoms (Aim 1).

Second, we investigated the structure of relationships among the symptoms of PGD and their relationships to symptoms of PTSD and depression with network analyses. Interest in intersymptom relationships has grown in recent years with some researchers proposing that causal relationships among symptoms of a disorder may contribute to their tendency to cohere as a syndrome (Borsboom & Cramer, 2013). From this perspective, PGD symptoms are not passive indicators of an underlying disorder, but components of a causal system capable of affecting and sustaining one another (Maccallum, Malgaroli, & Bonanno, 2017; Robinaugh, LeBlanc, Vuletich, & McNally, 2014), a possibility according with theories of PGD (Boelen, Van Den Hout, & Van Den Bout, 2006). Moreover, the high comorbidity observed among psychiatric disorders, such as PGD, PTSD, and depression, may be attributable to causal relationships among their constitutive symptoms (Cramer, Waldorp, van der Maas, & Borsboom, 2010; Maccallum et al., 2017; Malgaroli, Maccallum,

& Bonanno, 2018; Robinaugh et al., 2014). However, no study has jointly examined the network structure of relationships among PGD, PTSD, and depression symptoms. In this study, we address this need by investigating the intersymptom relationships that bridge these syndromes and, thus, potentially contribute to their tendency to co-occur (Aim 2).

There is evidence that a symptom-level approach may provide new insights into how external factors relate to mental disorders. For example, research in depression has shown that individual symptoms exhibit distinct patterns of association with risk factors, neurobiological activity, and psychosocial impairment (Fried et al., 2015). These symptom-specific associations provide clues to the paths by which these factors affect or are affected by the broader syndrome. Here, we used this approach to investigate the relation of two types of bereavement (violent loss and the loss of a partner or child) with the clinical picture presented by this population. We did so by (a) investigating the general symptom network structure in those with and without the factor, (b) comparing mean symptom severities of those with and without the factor, and (c) examining associations between the factor and specific symptoms of PGD, PTSD, and depression (Aim 3).

2 | METHODS

2.1 | Participants and procedure

Participants were patients referred to Foundation Center '45, a specialized Dutch clinic for the diagnosis and treatment of PTSD and related disorders who reported to have lost a loved one in the routine outcome measurements (ROM). Participation for ROM is voluntary for both the patient and therapist. In total, 1,572 patients were admitted in the period of data collection (March 19, 2015 to July 17, 2017); 642 (41%) patients reported bereavement on the ROM. Of these patients, 458 (71%) completed the three questionnaires used in the current study within the first 3 months after admission. Following a consultation by the medical ethics committee of Leiden University in the Netherlands, the study was exempted from formal review because the primary purpose of the ROM is diagnostic and not research oriented. All patients were informed during the ROM that their answers could be anonymously used for research purposes and could object if they did not agree.

2.2 | Questionnaires

All participants completed three questionnaires: The Traumatic Grief Inventory Self Report version (TGI-SR; current sample $\alpha = 0.95$; Boelen & Smid, 2017), the PTSD Checklist (PCL-5; $\alpha = 0.93$; Blevins, Weathers, Davis, Witte, & Domino, 2015; Boeschoten, Bakker, Jongedijk, & Olff, 2014), and the Brief Symptom Inventory (BSI) Depression subscale ($\alpha = 0.95$; Derogatis & Melisaratos, 1983). We examined a subset of core symptoms of PGD, PTSD, and depression to keep the number of estimated parameters in proportion to our sample size. For PGD, we used items three to 12 of the TGI-SR questionnaire, corresponding to diagnostic criteria proposed by Prigerson et al. (2009). For PTSD, we selected items corresponding to the six-item PCL (items 1, 4, 7, 13, 15,

TABLE 1 Questionnaires and descriptions of symptoms

TGI-SR PGD items Prigerson et al., 2009	BSI Depression items Derogatis & Melisaratos, 1983	PTSD PTSD checklist items Lang & Stein, 2005
Yearning	Thoughts of ending your life	Memories, thoughts, or images
Role confusion/ diminished sense of self	Feeling lonely	Upset when reminded
Difficulty accepting the loss	Feeling blue	Avoid activities or situations
Avoidance of the reality of the loss	No interest in things	Feeling distant or cutoff
Experiencing mistrust/inability to trust others since the loss	Feeling hopeless about the future	Irritable or angry
Bitterness over the loss	Feelings of worthlessness	Difficulty concentrating
Difficulty moving on with life		
Feeling emotionally numb since the loss		
Feeling life is empty or meaningless		
Feeling stunned, dazed or shocked by the loss		

Note. BSI, Brief Symptom Inventory; PTSD, posttraumatic stress disorder; PGD, prolonged grief disorder; TGI-SR, Traumatic Grief Inventory Self-Report.

and 19), representing symptoms from each of the DSM-IV symptom clusters (Lang & Stein, 2005). For depression, we used the full BSI depression subscale (i.e., items 9, 16, 17, 18, 35, and 50; Derogatis & Melisaratos, 1983) (Table 1, Figures 1–4).

2.3 | Statistical analysis

For Aim 1, we used LCA with Mplus version 8 (Muthén & Muthén, 1998–2011) to identify classes of bereaved individuals with comparable PGD, PTSD, and depression symptom profiles. All items were dichotomized with the highest three-values on a five-point scale signifying endorsement (cf. Boelen, Reijntjes, Djelantik, & Smid, 2016; Djelantik et al., 2017b; Maccallum & Bryant, 2018; Nickerson et al., 2014). We began with a one-class model and increased the number of classes until we reached the most parsimonious and best-fitting model.

For Aim 2, we used network analysis to examine relationships among symptoms of PGD, PTSD, and depression. Our networks comprised two components: symptoms of PGD, PTSD, and depression, represented as nodes in the networks, and the regularized partial correlations between symptoms, represented by a link between nodes (referred to as an "edge"). All network analyses were conducted using R (R Core Team, 2013). We estimated the network and assessed the precision of edge weight estimates and stability of centrality indices using the bootnet package (v. 1.0.1). We visualized the network using the package qgraph (v. 1.4.4). We then used the spinglass algorithm

from the R package igraph (v. 1.2.2) to examine the community structure of the network. Because the algorithm is nondeterministic, we used a recently developed iterated community detection procedure to perform the analysis 10,000 times and examine the consistency of results across iterations (Werner, 2018). As a sensitivity analysis, we used a second community detection algorithm (infomap) and evaluated the convergence of the findings with the results of the spinglass analysis.

For Aim 3, we examined mechanisms by which two loss-related factors (violent loss and the loss of a partner or child) may contribute to this symptom network. These analyses were completed only for participants who reported on these loss-related characteristics (n = 400). First, we examined whether the structure of the network differed as a function of violent loss using the package NetworkComparisonTest (v. 2.0.1) (van Borkulo et al., 2018). This test needs comparable sample sizes for the groups being compared, preventing us from conducting this test for the "parent/child loss" variable. Then, we conducted symptom severity analyses. Finally, we used the package mgm (v. 1.2-3) to assess the regularized partial correlations between loss characteristics and individual symptoms.

3 | RESULTS

3.1 | Sample characteristics

The final full sample comprised 324 (71%) men and 134 (29%) women with a history of traumatic experiences (M = 6.6, SD = 3.3) and lifetime losses (M = 3.6, SD = 2.1). Their average age was 49.1 (SD = 12.2) years. A total of 204 (45%) participants had a history of at least one violent loss. A total of 57 (12%) participants had lost a partner and/or a child. The proportions of patients with clinically relevant PGD, PTSD, and/or depression were 28% (cutoff: 61, n = 126), 78% (cutoff: 32, n = 359), and 51% (cutoff: 11, n = 235), respectively (Blevins et al., 2015; Boelen & Smid, 2017; Derogatis & Melisaratos, 1983). Compared to patients who did not complete all three questionnaires, the included patients were more likely to be older, to be born in the Netherlands, and to have experienced a profession-related trauma (Supporting Information A–C).
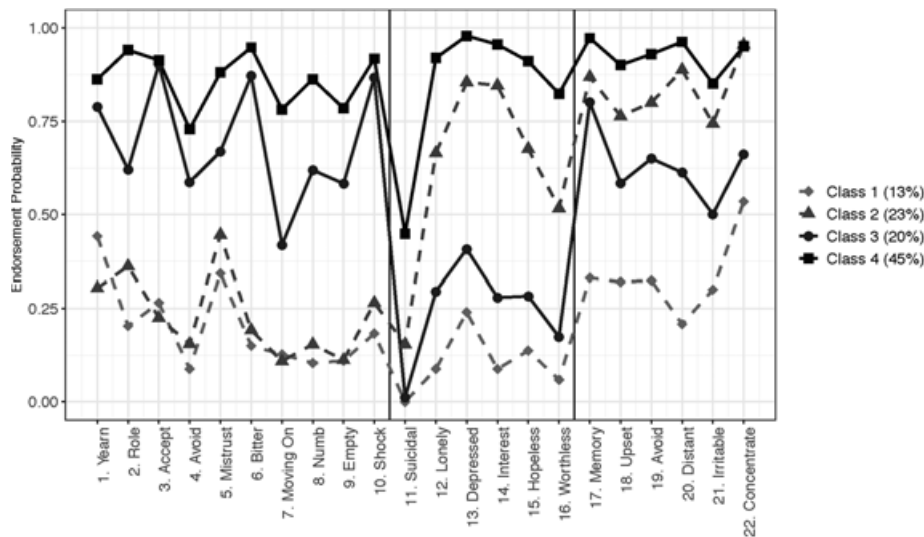
3.2 | Aim 1: Latent class approach

The three-class, four-class, and five-class solutions each yielded suitable models considering the goodness-of-fit statistics and smallest sample size (Table 2). However, the four-class solution had the best combination of interpretability (i.e., classes with clear high and low endorsement of symptoms), parsimony, and goodness-of-fit indices. We considered a value of >0.6 as a prominent probability of symptom endorsement (Maccallum & Bryant, 2018; Nickerson et al., 2014). The four-class solution consisted of a class of patients with no prominent PGD, PTSD, and depression symptoms (13%), a class of patients with prominent PTSD and depression symptoms (23%), a class of patients with prominent PGD and PTSD symptoms (20%), and a class of patients with prominent PGD, PTSD, and depression symptoms (45%) (Figure 1, Supporting Information D and E).

TABLE 2 Goodness-of-fit statistics for 1 to 6 class solutions

Classes	Log likelihood	BIC	SS-BIC	AIC	Entropy	BLRT	VLMRT	Smallest sample size (n)
One class	−6,179.049	12,492.888	12,423.067	12,402.097				
Two classes	−5,248.773	10,773.254	10,630.438	10,587.545	0.914	$P < 0.001$	$P < 0.001$	216
Three classes	−5,003.364	10,423.356	10,207.544	10,142.728	0.911	$P < 0.001$	$P < 0.001$	111
Four classes	−4,871.082	10,299.709	10,010.903	9,924.164	0.900	$P < 0.001$	$P = 0.57$	58
Five classes	−4,785.231	10,268.925	9,907.124	9,798.462	0.880	$P < 0.001$	$P = 0.13$	49
Six classes	−4,740.656	10,320.693	9,885.896	9,755.312	0.881	$P < 0.001$	$P = 0.33$	48

Note. In the six-class model, the BIC (Bayesian Information Criterion) started increasing again, so we did not test more models. BIC, Bayesian Information Criterion; SS-BIC, sample-size adjusted BIC; AIC, Akaike Information Criterion; BLRT, bootstrapped likelihood ratio test; VLMRT, Vuong-Lo-Mendell-Rubin test.

FIGURE 1 Symptom endorsement probability for the four-class solution of the latent class analysis

3.3 | Aim 2: Network approach

Network analyses (see Supporting Information F–M) suggested reasonable precision in the estimated edge weights and good stability of the strength and expected influence centrality indices (CS-coefficients = 0.59 and 0.67, respectively). Consistent with prior research (Epskamp, Borsboom, & Fried, 2018), we observed relatively low stability of the betweenness and closeness indices (CS-coefficients = 0.13 and 0.28, respectively).

The results of our spinglass community detection analyses appear in Figure 2. In 22% of iterations, the algorithm suggested three communities grouped along diagnostic boundaries while 75% of iterations identified four communities, most commonly including a community of depression symptoms, a community of PTSD symptoms, and two communities of PGD symptoms: one community defined by shock, bitterness, avoidance, and difficulty accepting the loss, and a second community defined by numbness, difficulty moving on with life, difficulty trusting others, and role confusion. The membership of yearning and emptiness oscillated between these communities. The infomap algorithm detected three communities along diagnostic boundaries in 100% of iterations (Supporting Information L). Together, these analyses suggest PGD, PTSD, and depression were distinguishable communities of symptoms with some evidence for two communities of symptoms within the PGD syndrome.

We next examined the intercommunity edges, considering each syndrome as a distinct community (Figure 3). We focus our interpretation only on the most robust intercommunity edges (those with confidence intervals in our bootstrap analysis that did not include zero). The depression symptom "loss of interest" was strongly associated with the PTSD symptoms "difficulty concentrating" and "irritability," consistent with these latter symptoms being part of the depression syndrome (Fried, 2017). Similarly, the PTSD avoidance and PGD avoidance symptoms were strongly associated. Across syndromes, associations were observed among symptoms that centered on themes of social disconnection and a negative sense of self-worth or purpose in life, including "feeling distant from others" (PTSD), "feelings of worthlessness" (depression), and "confusion about one's role in life" and "difficulty trusting others" (PGD). "Feeling distant from others" (PTSD) exhibited the strongest intercommunity expected influence.

3.4 | Aim 3: Risk factor-specific associations

The network comparison test indicated that both the structure of the network and its global strength did not differ significantly between
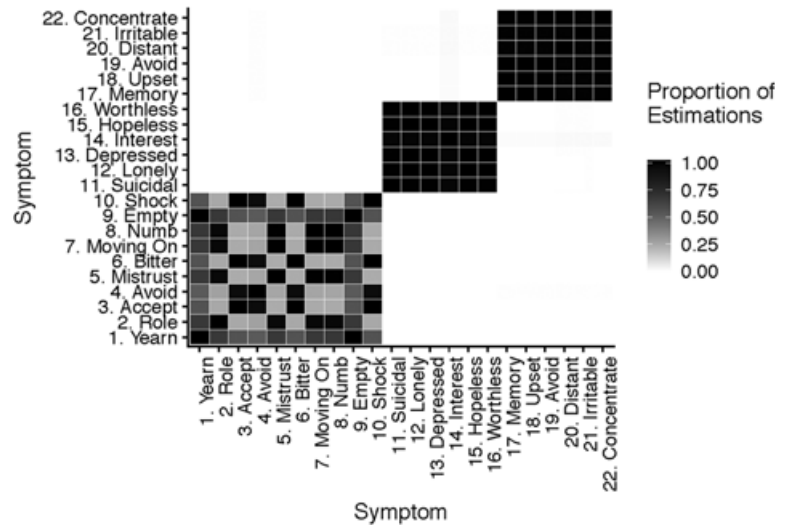
FIGURE 2 Analysis of shared community membership. Darker cells represent a higher proportion of the 10,000 iterations of the spinglass community detection algorithm in which the relevant symptoms appeared in the same community
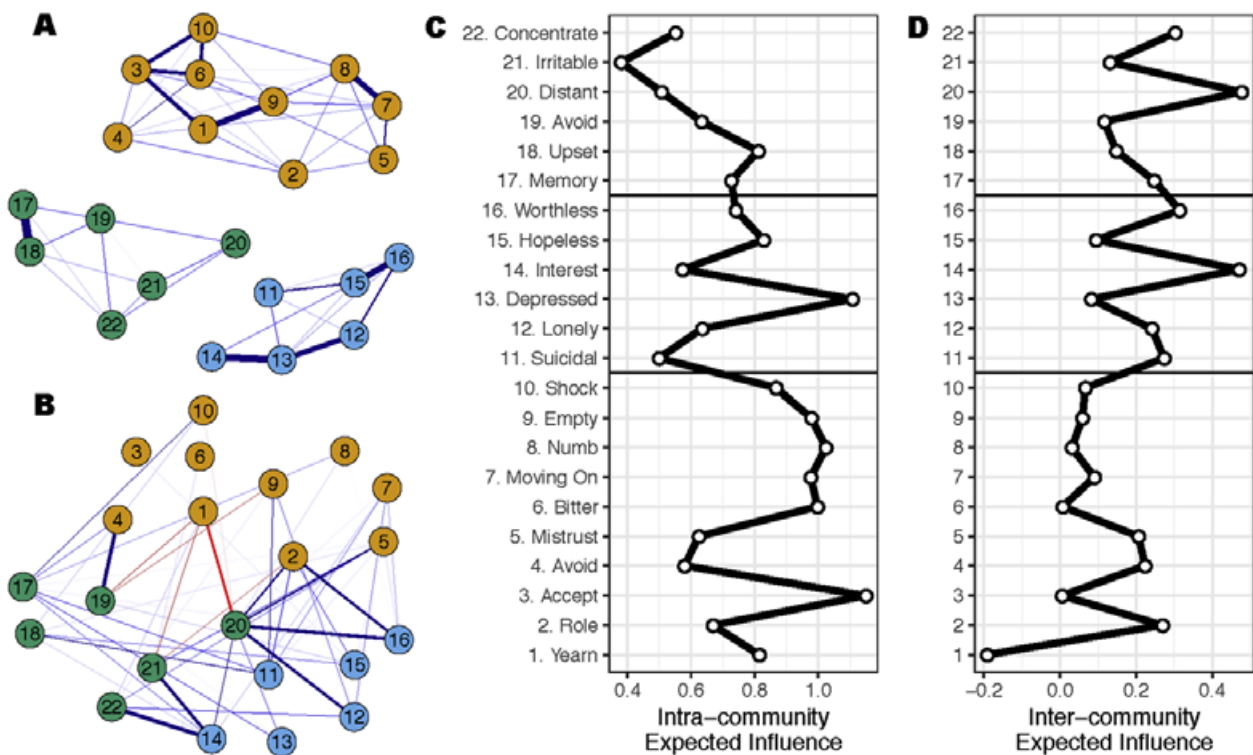

FIGURE 3 Analysis of intra- and intercommunity relationships. Intracommunity edges are depicted in panel (a). Intercommunity edges are depicted in panel (b). For both panels (a) and (b), blue edges represent positive associations and red edges represent negative associations. Thicker edges represent stronger associations. PGD, depression, and PTSD symptoms are represented by orange, blue, and green nodes, respectively. The intracommunity expected influence of each symptom (i.e., the sum of the edge weights between a given symptom and other symptoms of the same community) appear in panel (c). The intercommunity expected influence of each symptom (i.e., the sum of the edge weights between a given symptom and other symptoms of the other communities) appears in panel (d). Intracommunity edges (M edge weight $= 0.15$) tended to be stronger than intercommunity edges ($M = 0.04$)

those with versus without a history of violent loss (test statistic $M = 0.23$, $P = 0.28$ and test statistic $S = 0.22$, $P = 0.48$, respectively). In the symptom severity profile analyses, a violent loss was associated with significant elevations in all symptoms of PGD and depression, and PTSD symptoms "intrusive memories" and "emotional reactivity to intrusive memories." Experiencing the death of a partner or child was associated with a more circumscribed set of elevated symptoms, including yearning, emptiness, and difficulty with moving on in life.

To assess the unique associations between these factors and specific symptoms, we reestimated the network with each of these factors incorporated as a node in the network (Figure 4). A violent loss was most strongly associated with "difficulty accepting the loss." Experiencing the death of a partner or child exhibited weaker associations with symptoms of PGD, PTSD, and depression and was most strongly associated with "difficulty moving on in life."
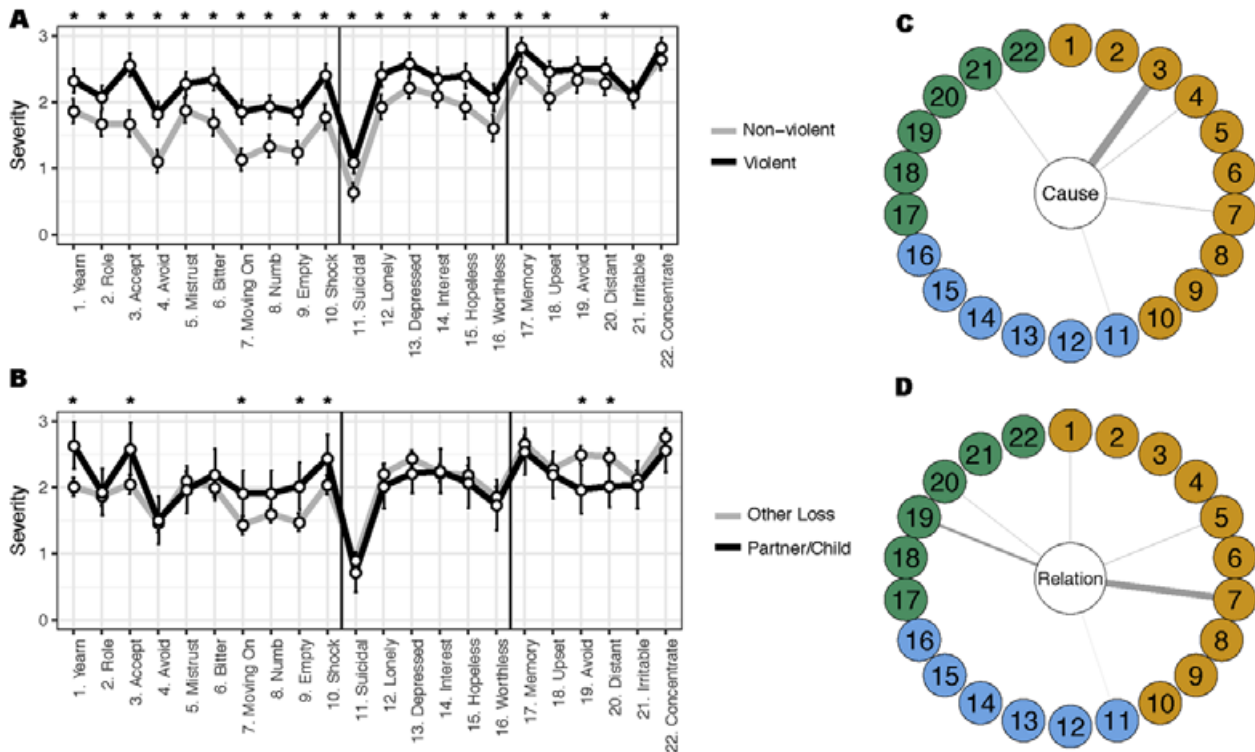
FIGURE 4 Analysis of loss-related risk factors and their association with symptoms of PGD, PTSD, and depression. Symptom profile plots between groups with and without a history of violent loss (a) and those who did versus did not lose a partner/child (b) identify symptoms elevated in those with each risk factor. Panels (c) and (d) depict the associations between these risk factors and symptoms of PGD, depression, and PTSD symptoms after controlling for all other symptoms. PGD, depression, and PTSD symptoms appear in orange, blue, and green, respectively. *$P < 0.05$

4 | DISCUSSION

This study is the first to investigate PGD, PTSD, and depression symptoms in a sample of treatment-seeking trauma-exposed patients and the first to jointly examine the relationships among these symptoms. Nearly half (41%) of all patients reported having experienced the death of a loved one. This number is a lower-bound estimate for the rate of bereavement as not all admitted patients completed this survey. The majority of our bereaved patient sample (65%) was member of a class that included some elevated endorsement of PGD symptoms and 20% were members of a class principally characterized by elevated PGD symptom endorsement and, to a lesser extent, elevated endorsement of PTSD symptoms. We did not identify a class characterized by elevated depression, comparable with the findings of the previous study in a community sample (Djelantik et al., 2017b). These results suggest that failing to assess PGD symptoms in this population would lead to an omission of a significant portion of the clinical picture presented by the majority of these patients and that it is important to improve treatment options for patients with comorbid PGD and PTSD (de Heus et al., 2017; Smid et al., 2015).

Second, we found that the symptoms of PGD, PTSD, and depression tended to hang together as distinguishable but highly interconnected communities of symptoms. We observed three types of overlap among these communities. First, there is overlap at the symptom level. For example, 'avoidance of reminders of the loss' and 'avoidance of the traumatic event' were strongly connected between PGD

and PTSD. Because these symptoms also have overlapping content, we might consider these symptoms as distinct indicators of the same phenomenon, especially in the case where the loss was used as the anchor event for the PTSD symptoms. Second, there was overlap at the syndrome level, with strong intercommunity relations observed involving symptoms commonly identified as a member of multiple syndromes. For example, we found that the depression symptom "loss of interest" was strongly associated with the PTSD symptoms "difficulty concentrating" and "irritability," a finding that is consistent with the identification of these symptoms as part of the broader depression syndrome. Third, we observed strong intercommunity relationships among distinct but closely conceptually related symptoms centered around feelings of self-worth, self-identity and social relationships (i.e., "feeling distant from others" (PTSD), "feelings of worthlessness" (depression), and "confusion about one's role in life" and "difficulty trusting others" (PGD)). These results accord with prior network analyses in community samples (Malgaroli et al., 2018; Robinaugh et al., 2014) and are reminiscent of theories stressing the importance of role-transition and meaning making in adjustment to bereavement (Maccallum & Bryant, 2013; Malgaroli et al., 2018; Neimeyer, 2016; Stroebe & Schut, 2001). Also, connectedness and positive sense of identity have been identified as themes in general recovery processes in people with mental health issues (Leamy, Bird, Le Boutillier, Williams, & Slade, 2011). Treatment programs targeting identity disruptions and meaning-making after the loss of a loved one have been found to be effective (Boelen, de Keijser, van den Hout, & van den Bout, 2007; Bryant et al., 2014; Shear et al.,

2016). Therefore, it may be that if an individual is not able to address the problems with social isolation and identity confusion, it will confer risk for other symptoms of PTSD, PGD, and depression, though this possibility remains highly speculative.

Concerning our third aim, we did not observe significant network differences between the groups in the network structure as a function of the nature of the loss (i.e., nonviolent vs. violent). This finding is consistent with prior work on PGD and PTSD, where comparable network structures were observed across different demographic characteristics (e.g., gender) and across different samples (Fried et al., 2018; Maccallum et al., 2017). However, recently, Benfer et al. (2018) showed that PTSD networks might differ between the types of trauma. Future studies need to elucidate this further. Both violent loss and the loss of a partner or child were more strongly associated with elevations in PGD symptoms than elevations in PTSD or depression symptoms, suggesting that it is especially important to assess PGD in these patient groups. Violent loss was most strongly associated with difficulty accepting the loss, which, in turn, was strongly associated with other PGD symptoms (e.g., a continued sense of shock, bitterness, and yearning; see Figure 3), suggesting that this symptom represents a mechanism through which violent loss contributes to other PGD symptoms and, subsequently PTSD and depression. Consistent with this possibility, we previously found evidence that PGD symptoms precede increases in PTSD symptoms over time in bereaved adults (Djelantik, Smid, Kleber, & Boelen, 2017a). In contrast, the loss of a partner or child was most strongly associated with the PGD symptom difficulty moving on with life, a finding consistent with prior reports that those experiencing the death of a partner are especially vulnerable to experiencing the belief that the future holds no meaning (Prigerson et al., 2009).

Generalization of our findings can only be done with caution because of the following limitations. Although larger than any prior analysis of the PGD symptom network, our sample remained smaller than ideal, given the number of estimated parameters, especially for the network comparison test (van Borkulo et al., 2018). To mitigate the limitation of sample size, we performed all analyses with only a constrained number of symptoms. Although doing so should afford more stability in our analyses, it also precluded inference about the omitted symptoms. Moreover, the observed associations may differ in networks that include additional symptoms. Accordingly, the observed findings must be interpreted in the context of the symptoms we included in the network. It should also be noted that we observed several very strong zero-order correlations between symptoms (Supporting Information F), which can lead to instability in the estimation of regularized partial correlations. To address this concern, we limited our interpretation of the network to those features that were found to be most robust in our bootstrap analyses. Finally, community detection algorithms remain new to psychological networks and, thus, should be interpreted with some caution. Nonetheless, the robustness of these findings across iterations and algorithms permits some confidence in our results.

Lastly, PTSD symptoms were reported in regards to a wide range of traumatic events. As a result, subjects could, but were not required to, report PTSD symptoms in regard to the death of a loved one. When PTSD symptoms are reported in regard to bereavement, endorsement

of some PGD symptoms (e.g., avoidance of thoughts about the death) would seem to necessitate endorsement of some PTSD symptoms (e.g., avoidance of thoughts about the trauma). This could result in stronger relationships between these symptoms in the network and more predominant comorbid classes in the LCA.

In conclusion, in this study among treatment seeking traumatized individuals, we found that bereavement and PGD symptoms were common and highly interconnected with PTSD and depression. These findings suggest that clinicians and researchers working with trauma-exposed populations should be aware of bereavement and PGD symptoms in order to adequately capture the clinical presentation of these patients.

AUTHOR CONTRIBUTIONS

M.D., P.B., and G.S. were responsible for the data collection. M.D. was responsible for the design of the study. M.D. and D.R. were responsible for the data analysis and interpretation of the data. D.R. and P.B. supervised M.D. M.D. wrote the drafts of the manuscript. D.R., G.S., R.K., and P.B. were involved in revising the draft versions critically and all authors approved the final version of the manuscript.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.