General discussion
Major Depressive Disorder (MDD) is a highly heterogeneous disease; subgroups of patients with MDD may differ considerably in their clinical presentation and likely may have differential biological correlates. This heterogeneity of the current MDD diagnosis may thus hinder research on risk factors and biological markers, and thus hamper progress in depression research and new developments in depression treatment. Substantial evidence has suggested that anxious depression is one such clinically important, more homogeneous entity of MDD [1,2]. However, various definitions for anxious depression are being used across studies, making results not always directly comparable. New developments in the field of anxious depression may contribute to a more uniform, clinically relevant definition, as the DSM-5 includes a new anxious distress specifier [3]. The main objective of this thesis was to establish whether anxious distress in depression is a distinct and clinically important depressive symptom profile. This objective was addressed in this thesis from different perspectives.

Summary of the main findings

Chapter 2 examined first the discriminant performance and convergent validity of the DSM-5 anxious distress specifier. Next, the longitudinal predictive validity of the anxious distress specifier for clinical outcomes was tested, and validated against comorbid DSM-IV-based anxiety disorder diagnoses. This was done in a large cohort of 1,080 patients with current (6-month recency) MDD at the NESDA baseline assessment. The analyses revealed that the anxious distress specifier was present in half of the patients with MDD (prevalence=54.2%). Also, the anxious distress specifier significantly discriminated in clinical characteristics, had convergent validity for anxiety characteristics and poorly overlapped with DSM-IV-based anxiety disorder diagnoses (Cohen’s $\kappa$=0.09). Interestingly, patients with the specifier had more often an MDD diagnosis after 2 years, a longer time to remission of their MDD diagnosis, and a higher functional disability after 2 years than those without the specifier. Moreover, the predictive value of the anxious distress specifier was better than that of the presence of a comorbid anxiety disorder diagnosis for these clinical outcomes. These findings suggest that the short anxious distress specifier outperforms comorbid DSM-IV-based anxiety disorder diagnoses as a longitudinal predictor for clinical outcomes in patients with MDD.

After establishing that the DSM-5 anxious distress specifier is a valid measure to predict subsequent clinical outcomes, Chapter 3 tested the ability of the DSM-5 anxious distress specifier to predict subsequent treatment outcomes (depression severity after 1 year and 2 years, and remission of MDD after 2 years) and frequency of antidepressant side effects in 149 patients with new-onset MDD on recently initiated adequate antidepressant treat-
ment. The predictive value of the specifier for treatment outcomes was also compared to that of comorbid DSM-IV-based anxiety disorders. The analyses further revealed that the specifier significantly predicted higher depression severity after 1 year and 2 years, lower remission rates after 2 years and greater frequency of antidepressant side effects during treatment. In contrast, the presence of comorbid anxiety disorders did not predict these treatment outcomes. Thus, the findings presented in this chapter suggests that the simple 5-item anxious distress specifier may be clinically relevant and useful for treatment planning and monitoring in patients with depression, as depressed patients with anxious distress have poorer treatment outcomes than those without anxious distress.

The previous chapters revealed that the anxious distress specifier is a clinically valid construct to predict both clinical and treatment outcomes in patients with current MDD. Therefore, it is important to examine whether the anxious distress specifier is characterized by an underlying biological profile, which could contribute to its prediction of poor clinical and treatment outcomes. In Chapter 4 it was investigated whether anxious distress and related anxiety dimensions were associated with differential immune dysregulation in a large cohort of 1,078 patients with current (6-month recency) MDD. To establish this, several basal inflammatory markers (C-reactive protein [CRP], interleukin [IL]-6, tumor-necrosis factor [TNF]-α, and a basal inflammation index score) and innate cytokine production capacity (13 lipopolysaccharide [LPS]-stimulated inflammatory markers and an LPS-stimulated inflammation index score) were examined. It was found that basal inflammation was not associated with anxious distress (prevalence=54.3%) in patients with current MDD, except for a modest positive association for one of the dimensional anxiety constructs (Beck Anxiety Inventory [BAI] score). Interestingly, anxious distress was however associated with higher LPS-stimulated inflammation levels (interferon [INF]-γ, IL-6, monocyte chemotactic protein [MCP]-1, macrophage inflammatory protein [MIP]-1α, matrix metalloproteinase [MMP]-2, TNF-α, LPS-stimulated inflammation index). Other dimensional anxiety indicators (anxious distress specifier score, BAI, Mood and Anxiety Symptoms Questionnaire Anxious Arousal subscale [MASQ-AA]) were also associated with increased innate production capacity. These results provide new insight into the pathophysiology of anxious depression as the anxious distress specifier was associated with increased innate cytokine production capacity but not with basal inflammation, which was largely confirmed by results from dimensional anxiety indicators.

Chapter 5 included a comprehensive review of anxious depression (MDD with a comorbid anxiety disorder or with significant anxiety symptoms) including anxious distress in depression, and reflected on the value and place of anxious distress in depression within the field of anxious depression. This review provided an overview of the latest
literature on the impact of co-occurring anxiety in patients with depression on clinical course trajectories and outcomes, treatment outcomes, and its possible underlying neurobiological dysregulations. Based on the latest literature, anxious depression seems to be common (42–78%) and associated with poorer clinical and treatment outcomes than ‘pure’ depression, despite heterogeneity in diagnostic criteria and assessment instruments. As populations with anxious depression may benefit from novel or more intensive treatment strategies, research efforts have been made to identify specific efficacy of novel pharmacological treatments for patients with anxious depression. Nevertheless, these studies have not been conclusive due to the insufficient number of studies and heterogeneity in anxious depression definitions and assessment. With regard to neurobiology, anxious depression seemed to be associated with increased immune dysregulation and brain abnormalities, including more severe cortical thinning and increased corticolimbic functional connectivity patterns, as compared with non-anxious depression. Overall, this review shows that anxious depression appears to be a common and clinically relevant subtype of depression as it predicts poorer course trajectories and treatment outcomes, and is associated with distinct neurobiological correlates. While various anxious depression definitions were related to these poorer findings, further research is required into which underlying anxiety features are driving associations with clinical outcomes and biology. The first findings on the relatively new DSM-5 anxious distress specifier seem promising, but this specifier should be further examined and compared against other anxiety constructs.

Chapter 6 investigated the role of anxious distress in MDD and also of other specific clinical depression characteristics on 6-year incidence of a wide range of common somatic diseases within a large cohort of persons with (n=968) and without (n=1,089) MDD. First, it was examined whether onset risks of common somatic diseases differed between persons with current (6-month recency) MDD and persons with remitted MDD (presence of a lifetime MDD diagnosis but without an MDD diagnosis or any anxiety disorder in the past 6 months) with healthy controls (no lifetime depressive and anxiety disorder). Second, the role of specific depression characteristics was examined, including depression severity, depressive symptom clusters, individual depressive symptoms and depressive subtypes (MDD with anxious distress, atypical or melancholic features). The incidence of five somatic disease categories (cardiometabolic, respiratory, musculoskeletal and digestive diseases, and cancer) was examined. The analyses, which were conducted in those persons who were initially free of the somatic diseases under study and which were adjusted for important lifestyle factors, revealed that persons with current MDD had an increased 6-year incidence of any somatic disease, cardiometabolic diseases, musculoskeletal diseases, and digestive diseases when compared to healthy controls. Persons with remitted MDD also showed increased incidence risks of somatic diseases, but these results were
statistically non-significant. It was also found that a higher 6-year somatic disease incidence was associated with higher depression symptom severity, as well as with depressive mood symptoms and depressive somatic/vegetative symptoms. Depressive subtypes did not substantially differ in their somatic disease incidence risks. There was no significant difference in 6-year incidence rates of somatic diseases between current MDD patients with and without anxious distress versus controls. Both the group of MDD persons with and the group without anxious distress had comparable, significantly increased incidence risks than controls for any somatic disease (marginally significant for those with anxious distress), cardiometabolic, musculoskeletal, and gastrointestinal diseases. This chapter showed that persons with current MDD had a substantially elevated 6-year incidence risk of a multitude of common somatic diseases as compared to healthy controls, which was not restricted to one single somatic disease category. Higher depression severity was associated with a higher somatic disease incidence risk in a dose-response manner, and seemed to be mainly driven by mood and somatic/neurovegetative symptoms.
Discussion of the main findings

Depression and anxiety are closely related [4] and this has been acknowledged for a long time [5]. For instance, in the work of Robert Burton, ‘The anatomy of melancholy’, published in 1621, depression and anxiety have been acknowledged as melancholia which could encompass anxiety [5]. In the recent past, presence of subthreshold symptoms of both anxiety and depression that do not meet criteria of a depressive and anxiety disorder diagnosis (mixed anxiety-depressive disorder [MADD]) were proposed as a focus point for research and MADD criteria were added to appendix B of the DSM-IV [1,6]. However, the clinical relevance of MADD has shown to be insufficiently reliable and was therefore omitted from the DSM-5 [7]. To acknowledge the clinical significance of prominent anxiety features in MDD, DSM-5 included the DSM-5 anxious distress specifier. The DSM-5 anxious distress specifier was derived from the study by Goldberg et al. [8] which was conducted in 15 general medical or primary care clinics across 14 different countries. In this study, a brief five-item anxiety scale was established by item-response theory analyses to facilitate clinicians with the diagnostic assessment of anxious depression [8]. Symptoms of generalized anxiety from the Composite International Diagnostic Interview-primary care (CIDI-PC) [9] were used, which is an adapted version of the CIDI. The CIDI-PC differs from the CIDI in that the CIDI-PC assessed current symptoms in addition to the usual probes, the anxiety section does not have ‘skip outs,’ and that the duration of anxious symptoms was recorded to the nearest month (with one month being the shortest duration). While the DSM-5 is one of the classification systems that are commonly used by clinicians worldwide, empirical evidence on the anxious distress specifier lacked at the time of inclusion in the DSM-5. Clearly, there is a pressing clinical need to obtain empirical evidence on this anxious distress specifier, to investigate the validity of the anxious distress specifier, and to determine the specifier’s value beyond the identification of anxiety disorder diagnoses.

Aim: Is anxious distress in depression a distinct and clinically important depressive symptom profile?

In order to answer this main question, several sub aims were defined. The first sub aim was to establish how well the longitudinal predictive validity of the DSM-5 anxious distress specifier is for clinical outcomes in patients with current MDD, and how the specifier’s predictive validity is compared to that of comorbid DSM-IV-based anxiety disorder diagnoses. The second sub aim was to examine the ability of the DSM-5 anxious distress specifier to predict treatment outcomes and frequency of antidepressant side effects in patients with new-onset MDD on adequate antidepressant treatment, and how the specifier’s predictive validity is compared to that of comorbid DSM-IV-based anxiety disorder diagnoses for treatment outcomes. The third sub aim was to determine
whether anxious distress and other related dimensional anxiety scales were associated with differential basal inflammatory markers and innate cytokine production capacity in patients with current MDD. The fourth sub aim was on the exploration of the value and place of anxious distress in depression within the field of anxious depression, and considered clinical course trajectories and outcomes, treatment outcomes, and its possible underlying neurobiological dysregulations. Finally, the last sub aim was to examine the role of anxious distress in MDD and of other specific clinical depression characteristics on 6-year incidence of common somatic diseases.

**Validity of the DSM-5 anxious distress specifier**

To establish whether a measure is valid, several psychometric properties can be examined. In this thesis, we tested the specifier’s reliability (internal consistency and inter-item correlations), discriminant performance, convergent validity and longitudinal predictive validity for subsequent clinical and treatment outcomes. We found that the specifier’s internal consistency was moderate (Cronbach’s $\alpha=0.71$), and the inter-item correlations of the proxy items for the specifier were all statistically significant. In addition, the anxious distress specifier had substantial discriminant performance because it adequately discriminated for several clinically important depression characteristics. Patients with MDD with versus without anxious distress had a higher depression severity, a longer duration of their depression, a higher functional disability and a higher suicidal ideation.

Convergent validity measures how closely the construct is related to other variables and other measures of the same construct to which it should be related [10]. The specifier showed adequate convergent validity, as all of the examined anxiety characteristics were higher in depressed patients with the specifier than those without. Finally, the specifier showed sufficient longitudinal predictive validity for clinical outcomes, as depressed patients with versus without the specifier had lower remission rates of MDD after 2 years, had a longer time to remission of MDD, and showed higher functional disability after 2 years. The specifier also adequately predicted poor treatment response. In patients with new-onset MDD who received adequate antidepressant treatment, the specifier significantly predicted higher depression severity after 1 year and after 2 years, lower remission rates of MDD after 2 years, and greater frequency of side effects during treatment. Moreover, the anxious distress specifier was a better longitudinal predictor than presence of comorbid DSM-IV-based anxiety disorder diagnoses for both clinical course and treatment outcomes.

These findings are in line with the study by Zimmerman et al. [11] that also examined the validity of the anxious distress specifier [11]. In this study a self-report measure was developed to assess the specifier, and this self-report measure was found to be reliable and valid [11]. Our item-total correlations of the anxious distress specifier were slightly lower
than those of Zimmerman [11], which may be the result of the difference in used proxy items regarding concentration. Nevertheless, this suggest that our proxy of the specifier reflects the same concept of that from Zimmerman [11]. However, in Zimmerman's study more than two-thirds of the patients met the anxious distress specifier compared to half of the patients in our study. Since the sample of Zimmerman is comparable to our sample, this difference in prevalence of the specifier might be explained by the content difference. However, the Zimmerman study provided no longitudinal data, nor was the specifier's validity tested against the validity of comorbid anxiety disorders. In a recent field study conducted across four countries, the short five-item anxiety scale which was derived from the study of Goldberg et al. [12] was found to be clinically useful as it was able to adequately identify current anxiety in the majority of individuals in which primary care physicians suspected psychological distress [12]. It was suggested that this short anxiety measure is a realistic alternative to complex diagnostic algorithms used in specialised care settings. Another recent study showed that a measure of the DSM-5 anxious distress specifier, the DSM-5 anxious distress specifier interview (DADSI), is a reliable and clinically valid construct [13], and that it was as valid as the Hamilton Anxiety Scale (HAM-A) in measuring anxiety in depression [13]. The HAM-A is a reliable and valid interviewer-administered instrument assessing anxiety severity. However, the HAM-A was more highly confounded with depression measures than the DADSI [13]. Also, the DADSI is much shorter than the HAM-A. Therefore, short screening and diagnostic scales developed for anxiety assessment such as the anxious distress specifier may be clinically useful, as they are more feasible and easy to incorporate in clinical practice, as opposed to formal diagnostic assessment of anxiety disorders which requires thorough, time-consuming interviews. To date, there are no other studies at the outset of this thesis that have examined the longitudinal predictive validity of the anxious distress specifier for treatment outcomes. Our study on treatment outcomes is thus the first study showing that the anxious distress specifier has adequate predictive value for subsequent treatment outcomes in depressed patients receiving adequate antidepressant treatment. Taken together, the anxious distress specifier seems to be a reliable and valid construct that can adequately predict course and treatment outcomes in persons with current MDD.

Validity of the anxious distress specifier beyond that of comorbid anxiety disorder diagnoses

While various definitions of anxious depression, including anxious distress in MDD, were related to a poorer course and clinical outcomes, more research is needed to identify which underlying anxiety features captured in different anxious depression definitions are driving associations with clinical outcomes and biology. However, this thesis showed that the anxious distress specifier outperforms comorbid DSM-IV-based anxiety disorder diagnoses (one of the definitions of anxious depression) as a longitudinal predictor for important clinical and treatment outcomes in patients with MDD. Overlap between the
anxious distress specifier with formally diagnosed comorbid anxiety disorders was poor (Cohen’s κ=0.09), which indicates that the specifier may capture patients with MDD suffering from prominent anxiety features predictive of poorer outcomes, who may not be fully captured by the DSM-based anxiety disorder diagnoses. Of the anxious distress criteria, four out of five symptoms are typical for Generalized Anxiety Disorder (GAD) and one for Panic Disorder [14]. However, we found that the anxious distress specifier poorly overlapped with all types of anxiety disorders (Social Phobia, Panic Disorder with or without Agoraphobia, Agoraphobia and GAD) and thus did not show specificity with GAD and Panic Disorder. This indicates that the specifier is a rather generic marker of anxiety and seems to pick up significant anxiety symptoms that are not captured by comorbid anxiety disorders. Possibly, one or more of the anxious distress symptoms may be transdiagnostic factors (factors that capture transdiagnostic variance between disorders) underlying a concept that spans across depression and anxiety and this concept may be characterized by a distinct underlying neurobiological profile which accounts for a poor course and poor outcomes. Also, anxious distress symptoms as transdiagnostic factors may drive disorder persistence, and this may also be an explanation for a poor course and poor clinical outcomes. Another possibility is that one or more of these rather generic anxious distress symptoms, that are not fully captured by specific types of comorbid anxiety disorders, account for ‘centrality’ (a measure for the most connected, clinically relevant, and maintaining symptoms in a symptom ‘network’), and thus may maintain the presence of overall depressive symptoms leading to a poor course and poor outcomes. In addition, the anxious distress specifier only assesses presence of several anxious symptoms that have to be present during the majority of a depressive episode, regardless of other aspects such as the context in which these symptoms occur. Contrary, diagnostic criteria for anxiety disorders are much more extensive and differ per type of anxiety disorder. For instance, one of the criteria for a Social Phobia is that anxiety must follow on social interactions, and in the case of a Panic Disorder the anxiety has to be related to worry about having a panic attack or about its consequences. It may be possible that patients with MDD and a comorbid anxiety disorder suffer less from additional anxiety as provoking factors might not evolve frequently—particularly when these factors are strongly avoided by patients—, whereas patients with MDD meeting criteria for the specifier generally suffer from anxiety symptoms most of the time. Another possible explanation for the better predictive validity of the specifier as compared to that of anxiety disorder diagnoses for clinical and treatment outcomes may be that the persistent presence of anxious distress may lead to a poor course and to poor clinical outcomes. Up until now, to our knowledge, no other studies than ours have tested the predictive validity of the specifier against that of comorbid anxiety disorders. This thesis provides preliminary evidence that the DSM-5 anxious distress specifier is of clinical value beyond of comorbid anxiety disorder diagnoses.
**Anxious distress or depression severity?**

A pressing question in the interpretation of the results on the anxious distress specifier is whether these results are specifically due to anxious distress in depression or are rather due to increased depression severity. In order to rule out potential confounding effects of depression severity, it seems necessary to correct for depression severity in the analyses on the anxious distress specifier. The value of adjusting for severity is unclear, given that patients with MDD meeting criteria for the anxious distress specifier already have higher depression severity by definition (i.e. more symptoms are required). In addition, it has been suggested that anxiety may be an epiphenomenon of the more severe forms of MDD, rather than a distinct phenomenon [15]. Therefore, we consider correction for depression severity potentially as an over-correction. However, as correction for severity can still be informative when interpreting results, we applied correction for depression severity in the analyses conducted in this thesis.

We corrected for depression severity in several different ways. First, in our study that examined the predictive validity for clinical outcomes, we stratified by depression severity, in which non-severe and severe groups were created based on the median IDS score. These stratified analyses showed no striking differences between the stratified effect sizes for the dichotomous specifier indicator. Overall risk estimates were largely similar in the non-severe and in the severe group. Stratified analyses for the continuous anxious distress indicator also showed equal predictive validity in both the non-severe and severe subgroups. These stratified analyses overall suggest that the anxious distress specifier has equal predictive validity across different severities of illness. Second, in our study on treatment outcomes, we adjusted for depression severity based on the number of DSM criteria endorsed. A priori, we considered the number of MDD criteria endorsed as the simplest measure of depression severity. We based this on the fact that patients with MDD plus the anxious distress specifier have to endorse a higher number of symptoms by definition—both MDD criteria and anxious distress features—than MDD patients without the specifier. In addition, the number of DSM MDD criteria endorsed is also a more clinical approach than a dimensional score such as the Inventory of Depressive Symptomatology (IDS) score. Although we considered use of the IDS to define severity, this in itself could be problematic in that some items of the specifier were derived from the IDS, and we therefore chose an independent measurement of severity. As expected, the number of DSM-IV MDD criteria endorsed was significantly higher among MDD patients with the specifier than without the specifier, even though the range of this measure is indeed relatively small. The specifier significantly predicted poor treatment outcomes, also after additional adjustment for depression severity. Finally, we also adjusted the analyses on inflammation for depression severity in an additional step based on the Quick Inventory of Depressive Symptomatology (QIDS) total score without the overlapping
anxious distress specifier items. After additional adjustment for severity, some of the positive associations between presence of the specifier and higher levels of LPS-stimulated inflammatory markers in MDD patients diminished to non-significant. However, the overall LPS-stimulated inflammation index was still significantly associated with anxious distress after adjustment of depression severity.

Thus, we examined the effect of depression severity in several different ways by correcting for different measures of depression severity, and demonstrated that the effect of depression severity on the anxious distress findings was small regardless of which severity measure was used. This suggests that the findings on anxious distress in MDD are not mainly driven by depression severity but likely relies on anxiety aspects.

**Prevalence rates of anxious distress in depression**

The prevalence rate of anxious distress was 54% in our sample of patients with current (6-month recency) MDD used throughout the studies in this thesis. This high prevalence rate is in line with prevalence rates of other studies that have examined anxious distress in MDD, which ranged from 56% to even 78% [11,16-18]. Methodological differences in sample characteristics and assessment instruments across these studies may have resulted in the somewhat higher rates of other studies. It should be noted that our high prevalence rates of anxious distress may have been an overestimation, as we used cross-sectional assessment of anxious distress criteria while the DSM-5 requires that anxious distress should be present during the majority of a depressive episode. Nonetheless, the study that found the highest prevalence rate of 78% established anxious distress by an anxious distress interview which also determined whether anxious distress was present during the majority of a depressive episode, indicating that even higher prevalence rates can be found when this criterion is taken into account. Also, anxious distress prevalence rates in MDD are in concordance with prevalence rates of anxiety symptoms based on other diagnostic assessments than the specifier in depression. This underlines the close relationship between depression and anxiety and its clinical relevance.

**Immune dysregulation as a pathophysiological mechanism of anxious distress in depression**

One of the pathophysiological mechanisms of depression that has been implicated is dysregulation of the immune system [19,20], as increased inflammation is found to be more often present in depression in several meta-analyses [21-23]. Inflammation is also implicated in the pathophysiology of treatment-resistant depression [24-26] and the pathophysiology of anxiety disorders [27-30]. It has been hypothesized that anxious depression could be seen as an inflammatory phenotype since it shares common pathophysiological pathways with inflammatory states [31]. At the start of this thesis, only two studies had examined the link between inflammation and anxious depression. One
study showed higher monocyte counts in patients with MDD and moderate-severe to severe anxious distress than in those with mild to moderate anxious distress [32]. The other study found decreased venous blood basophil counts and increased fragmented neutrophils in patients with MDD and high versus low levels of anxiety [33]. The findings of these studies reflect alterations in white blood cell subset counts, and indirectly may point to alterations in the immune system, suggesting the role of inflammation in the development of anxious depression.

We found higher innate cytokine production capacity in patients with MDD plus anxious distress, which was also confirmed by other indicators of high anxiety in MDD patients. Higher innate production was positively associated with anxiety indicators with a greater focus on somatic anxiety symptoms, which may imply that the association between anxious distress in MDD and higher innate production is mainly driven by somatic anxiety symptoms. This is in line with earlier research where somatic, but not cognitive, symptoms of anxiety and depression were associated with basal inflammation [34]. Moreover, somatic anxiety symptoms may reflect the hypervigilance characteristic of anxiety disorders—which was suggested to be part of the ‘pathogen host defense’ hypothesis where inflammatory systems are activated in response to stressors leading to the development of depression [19]—, thereby the results support this hypothesis. It is also possible that somatic anxiety symptoms are associated with increased sympathetic and decreased parasympathetic autonomic activity [35], since the autonomic nervous system is associated with higher inflammation levels [20,36,37]. Another explanation may be that inflammatory markers in the brain affect metabolic and molecular pathways that influence neurotransmitter systems (e.g. monoamines and glutamate) which ultimately affect neurocircuits regulating anxiety [19,38]. Alternatively, anxiety reflected as psychological stress, may induce inflammasome pathways leading to inflammation and ultimately to depression [19,39]. The innate inflammatory response is under strong genetic control [40] with heritability estimates ranging from 53–86% in non-diseased populations [40,41]. It may be possible that the anxious subtype of MDD has shared genetics with the innate production capacity. Future research should further examine this genetic link. Finally, another possible explanation for the found associations between higher LPS-stimulated levels and anxious distress in MDD patients may be that the inflammatory signals become amplified after ex vivo LPS-stimulation making it easier to find associations, rather than that basal inflammation and innate production capacity truly are being different immune concepts. Overall, the findings support the recent suggestion of the involvement of inflammation in the pathophysiology of anxious depression [33]. More specifically, the results support the hypothesis that patients with the anxious distress specifier may have different underlying biological profiles than patients without this specifier, which could explain earlier findings showing that the anxious distress specifier
predicts poorer course and treatment outcomes [42,43]. However, it should be noted that cross-sectional data was used in our study and therefore no causal inferences can be made. Also, the number of studies that have examined the link between inflammation and anxious distress in MDD is insufficient and findings should be replicated in order to draw firm conclusions. It is clinically relevant that research will build further on these findings to establish whether patients with MDD and anxious distress have a distinct underlying basis of immune dysregulation, because this subgroup may then specifically benefit from anti-inflammatory agents or new antidepressants with a target on inflammation.

**Somatic health consequences of anxious distress in depression and other depression characteristics**

It was found that persons with current MDD had a substantially elevated 6-year incidence risk of a multitude of common somatic diseases as compared to healthy controls, which was not restricted to one single somatic disease category. Of the depression characteristics, depressive subtypes (MDD with anxious distress, atypical or melancholic features) did not differ in their somatic disease incidence risks. Both the group of MDD persons with and the group without anxious distress had comparable, significantly increased incidence risks than controls for any somatic disease (marginally significant for those with anxious distress), cardiometabolic, musculoskeletal, and gastrointestinal diseases. This is inconsistent with one study that found an increased cardiovascular disease (CVD) onset risk for MDD patients with mild versus moderate-severe anxious distress, although lifestyle adjustment was not conducted [44]. Also, LCA-based severe atypical and severe melancholic subtypes versus controls were both significantly associated with a higher incidence of any somatic disease, cardiometabolic, musculoskeletal and gastrointestinal diseases (marginally significant for LCA-based atypical subtype). This indicates no specificity of subtypes for specific disease categories. This is surprising, as different depressive subtypes are linked to different biological dysregulations and may therefore be at risk for development of different somatic diseases. For example, atypical depression is associated with leptin dysregulations [45] and obesity [45,46], which possibly may contribute to developments of metabolic diseases. One study showed that atypical depression was prospectively associated with a higher incidence of metabolic syndrome and other cardiometabolic risk factors [47], and one other study recently showed that atypical depression is predictive of increased incidence rates of CVD compared to non-atypical depression [48]. We however, found that both the LCA-based melancholic and atypical depressive subtypes were associated with an increased incidence of cardiometabolic diseases. This could be because different biological dysregulations may all contribute to the onset of somatic diseases, but through a different pathway [49].
Conclusions aim:

Is anxious distress in depression a distinct and clinically important depressive subtype?
Based on the findings presented in this thesis on the relatively new DSM-5 five-item anxious distress specifier, this specifier seems to be a reliable and clinically valid measure that can adequately predict course and treatment outcomes in persons with MDD. Also, anxious distress in depression seems to have a distinct neurobiological profile with increased immune dysregulation and brain abnormalities, including more severe cortical thinning and increased corticolimbic-striatal functional connectivity patterns as compared to ‘pure’ depression. Nonetheless, anxious distress in MDD did not substantially differ in its somatic disease incidence risk. Overall, these findings indicate that anxious distress in depression appears to be a common, distinct, and clinically important depressive symptom profile.

The anxious distress specifier may be clinically useful to identify more homogeneous groups of patients with MDD and prominent anxiety features that may benefit from novel or more intensive treatment strategies. However, more research on the anxious distress specifier is required and should also be compared with other anxiety constructs.
Methodological considerations

The studies in this thesis provide important empirical evidence on the anxious distress specifier concerning validity, prognosis, treatment response and biological correlates within a large cohort of patients with current MDD; such evidence is relatively scarce in the current literature. The studies presented in this thesis also have several important strengths. The majority of the studies that were carried out were longitudinal, and therefore causal inferences on found associations can be made. Also, the analyses that were conducted for this thesis were adjusted for a broad set of important covariates, so that confounding effects could be eliminated. However, several methodological limitations about the studies in this thesis should be noted as well.

Diagnostic assessment of the DSM-5 anxious distress specifier

The proxy for the DSM-5 anxious distress specifier that was used in all studies presented, has several limitations. First, self-reported items from self-reported symptom questionnaires were used to construct this proxy of the anxious distress specifier, instead of using clinician-based assessments. Next, the item on concentration difficulties acquired from the IDS [50] is somewhat different than the DSM-5 anxious distress criterion for concentration, in that concentration difficulties due to depression may be different than those due to anxiety or anxiety disorders. However, our aim was to study the concept of anxious distress in MDD, rather than developing and validating an instrument to assess anxious distress. Although the content of our specifier differed slightly from the specifier in the study by Zimmerman and colleagues [11] (regarding the item on concentration), comparable reliability and validity results were obtained, suggesting that our proxy of the anxious distress specifier adequately reflects the DSM-5 anxious distress concept. Also, the cut-off used to determine presence of anxious distress specifier criteria was somewhat arbitrary. Nevertheless, we accounted for this by also examining a continuous indicator of the anxious specifier (number of specifier items or specifier total score) in addition to a dichotomous indicator; similar results were obtained for this continuous indicator. Finally, the anxious distress criteria were determined within the past week prior to the NESDA baseline assessment in persons with a current MDD diagnosis in the past 6 months of the baseline assessment. Since the timeframes of presence of anxious distress and current MDD do not fully overlap, one could argue that anxious distress may not have been present at the same time as the current MDD diagnosis, although this is required according to DSM-5. However, almost all (95%) of the patients who had an MDD diagnosis with a 6-month recency, showed evidence of recent depression activity as indicated by either the presence of MDD with a 1-month recency or an IDS score ≥14, indicating clinically relevant depression severity symptoms at the baseline assessment. Thus, it is highly likely that anxious distress and MDD were present at the same time.
Observational data for addressing treatment response

In this thesis we examined the predictive value of the DSM-5 anxious distress specifier for subsequent treatment outcomes in an adequately treated MDD sample using data from an observational cohort study. Ideally, the best design to examine a moderating effect of anxious distress would be a placebo-controlled randomized clinical trial (RCT) in which participants with new-onset MDD are randomized into an intervention (recent start of antidepressant or other novel drug) or control (placebo) arm. However, since we were able to only use observational data and had no control arm, we therefore carefully determined prior to conducting the analyses which approach best matched with an RCT design. In doing so, we succeeded to include patients with new-onset MDD episodes and a recent start of antidepressant treatment with an adequate dose and duration of use. The recent onset of both the MDD episode and the start of treatment make the assumption that the antidepressant medication was prescribed specifically for that particular MDD episode fairly strong. Also, long-term antidepressant users before baseline were excluded, as they were assumed to be chronic MDD patients. By using this approach, it is likely that only those persons were included in which treatment outcomes could be optimally determined.

Assessment of antidepressant medication use

Since initiation of antidepressant medication was one of the inclusion criteria in the study on treatment outcomes, it may be concerning that medication use was not additionally confirmed by the prescriber, that prescriptions may be inconsistent regarding dosages, and that medication intake may be unreliable. Nonetheless, all medication that was used in the month prior to the measurement was registered by inspection of the drug containers brought in by the participants, which was done by the majority (75.2%, n=112 patients) of the sample used in this study. Only when participants did not bring their drug containers, did we rely on patient self-report (which, if felt necessary, was also re-checked by later inspection of drug container labels during phone contact). Because all drug containers are provided with a label that shows the patient’s name, the prescriber’s name, frequency and dose of the drug as a standard procedure by pharmacies, we feel that drug container inspection was sufficient, without the need for additional confirmation from the prescriber. Medication intake as prescribed was additionally confirmed by participants during the interview. The inclusion criteria for frequency of use (i.e. ≥50% of the time), Defined Daily Dosages, and duration of use were determined based on these recorded data from drug container labels. With regard to the consistency of prescriptions, we can say the following: In the Dutch health care system, all healthcare professionals who provide mental health (General Practitioner [GP], Generalist Basic Mental Health Care, and Specialist Mental Health Care), provide care based on a uniform national guideline [51]. Since all patients in our sample were recruited from these three mental health
care settings, and all care professionals provided mental health care based on a uniform national guideline, general consistency in antidepressant prescribing is assumed. Also, reliability of medication intake/compliance is always difficult to measure in epidemiological studies and mostly relies on a patient’s self-report. We feel that by excluding patients reporting poor compliance, the remaining group of patients reflects adequate compliance. Considering all of the above, we believe that the registration of medication use in this study was valid and adequately reliable, employing the highest attainable method for an observational cohort study.

**Research within the diagnostic boundaries of the categorical DSM diagnostic classifications**

While one of the most widely used classification systems, the DSM, is of great value in clinical practice and enables a uniform language across clinicians worldwide, the DSM-based categorical classifications may not be suitable for all research purposes. All DSM-based classifications are based on clinical symptom presentation counts on which experts have reached consensus. These classifications do not incorporate information obtained from integrative research on pathophysiology or treatment response. Also, these classifications have a dichotomous nature, assuming that persons are either healthy or are suffering from a mental disorder. The clinical reality in which persons can have a depressive symptom severity ranging from mild to extreme is thereby disregarded. Moreover, while we can improve DSM-based psychiatric classifications by, for instance, identifying more specific symptom clusters or assess comorbidity of mental disorders within these diagnostic boundaries, diagnoses based solely on symptom presentation are likely heterogeneous in their pathophysiology. Indeed, many psychiatric symptoms can present across multiple mental disorders that may be characterized by unique underlying pathophysiological mechanisms that span across multiple diagnoses currently classified in the DSM. The anxious distress specifier was examined in this thesis within the diagnostic boundaries of the DSM classification of MDD. On the one hand, this is an advantage because anxious distress in MDD was strictly examined according to the DSM-5 definition and therefore provided evidence specifically on the MDD-anxiety concept included in the DSM-5. This may greatly contribute to the legitimization to use this anxious distress specifier in clinical practice. Also, it contributes to a better insight into the current MDD classification and partly reduces its heterogeneity. On the other hand, however, this approach can supply only a partial understanding of the MDD classification because the focus was only on one of the multiple putative depressive symptom profiles and the clinical application of this approach for treatment selection may be limited. So, conducting research only within the diagnostic boundaries of the DSM can result in loss of scientific information. Consequently, treatments may be limited only to symptom reduction and development of treatment interventions that target the possible underlying pathophysio-
logical mechanism may be hindered. Recently, novel approaches that are transdiagnostic and research-driven, have been proposed to overcome these possible concerns and to study the close relationship between depression and anxiety.

**Research based on a hypothesis-driven approach versus other novel transdiagnostic, data-driven approaches**

*Hypothesis-driven approach focusing on anxious distress in depression*

The research performed in this thesis was mainly based on a hypothesis-driven perspective in which we strictly examined a pre-defined DSM definition. Because anxious distress in MDD was hypothesized to be associated with poorer outcomes as compared to ‘pure’ MDD, the specifier was tested as a predictor for multiple important outcomes within patients with MDD. However, it should be noted that by using this approach, it was not examined whether specifically the anxious distress symptoms in relation to other symptoms, are truly driving the associations with poor findings. In addition, since we have shown that various definitions of anxious depression, including anxious distress in MDD, were related to a poor course and poor clinical outcomes, it is important to identify which underlying anxiety features captured in different anxious depression definitions are driving associations with clinical outcomes and biology. Although the anxious distress specifier has been derived by a more data-driven approach (IRT analysis), we examined this specifier by using a hypothesis-driven approach. Other novel approaches may also be useful to broader examine the close relationship of depression and anxiety taking into account the full anxiety/depression spectrum.

*Approaches based on the use of data-driven techniques*

*Data-driven latent variable model techniques*

An approach that may be useful to examine which anxiety symptoms are specifically important within patients with MDD, is one based on the use of several statistical data-driven techniques, also known as latent variable models. These models can recognize symptom patterns in depressive subgroups. Latent factor models and latent class models are the two major types of these models [54]. In addition, because symptom profiles based on a pre-existing hypothesis may only have a limited clinical application, more homogeneous depressive subtypes or dimensions across multiple disorders identified by data-driven techniques may be more valuable. Indeed, one study showed that when performing latent class analysis in a sample of persons with diagnoses of pure depressive episode, pure anxiety disorder, comorbid depressive episode and anxiety disorder, and MADD, the majority of all four diagnoses were included in one latent class [55]. This indicates that these conditions have distinct presentations, once the full profile of symptoms is taken into account. However, it should be noted that when performing such data-driven analyses, several aspects preceding the analyses are likely to influence the
retrieved classes or dimensions. For instance, the number, type and severity of included symptoms, the origin of the sample that may influence the severity of symptoms, the included number of patients, and model choice are all likely to influence outcomes [56].

**Statistical network analysis techniques**

Another promising approach to mental disorders is the use of sophisticated statistical network analysis techniques [57,58]. This network approach assumes that a causal interplay between psychiatric symptoms is the basis for psychopathology. It has been shown that overlapping symptoms in depressive and anxiety disorders as well as non-overlapping symptoms of depression and anxiety were connected [59,60]. A study by Fried et al. [61] examined a network of both DSM and non-DSM symptoms of MDD in large group of depressed patients. This study showed that the anxious and phobic symptoms, in addition to two core MDD symptoms, were among the most central symptoms, and thus accounts for the maintenance of the overall MDD symptom network [61]. It should be noted that patients included in that study were diagnosed based on the presence of these core depression symptoms, and this may have led to an overestimation of their centrality estimates. The network perspective may be a valuable approach to examine the spectrum of depression and anxiety, as it may provide a better insight into which symptoms mainly account for the comorbidity of depression and anxiety and into their underlying pathophysiological mechanisms. Targeting central symptoms (the most connected and clinically relevant symptoms in the network that also maintain the network) may thus result in an overall reduction in symptoms [58]. Most of the network techniques that have been used were cross-sectional and therefore conclusions on the temporality and direction of symptoms are hindered. Yet, innovative network techniques based on longitudinal data are emerging [62] and can be used to develop a directed network that reveals the direction of symptoms [58]. Taken together, the network approach appears to be one of the valuable approaches to offer better insights into the complexity of psychopathology and into more individualized symptom networks of patients that may inform tailored treatments [58].

**Approaches based on the use of the Research Domain Criteria framework**

The National Institute of Mental Health (NIMH), the major federal research agency funding mental health research in the United States, developed the Research Domain Criteria (RDoC) project to create a new, dimensional research framework [52,53]. This framework for organizing research intends to provide a novel transdiagnostic approach to investigate mental disorders. Multiple levels of data are integrated in order to develop a new approach to diagnostic classification that is based on pathophysiology, and to link it to specific treatments [53]. To organize research, an RDoC matrix was developed in which five major constructs (negative valence systems, positive valence systems, cognitive sys-
tems, systems for social processes, and arousal/modulatory systems) are presented in rows. These domains can be studied on seven different units of analyses (genes, molecules, cells, neural circuits, physiology, behaviors, and self-reports) which are presented in the columns. Research on different levels of neurobiology and behavior can thereby be integrated, and underlying causes of mental disorders can be elucidated, thus clarifying the boundaries and overlap between mental disorders. Of note, this framework does not consider important factors such as environmental exposures or longitudinal aspects of pathophysiology. If specific systems can be identified—using the RDoC approach—that span across both depression and anxiety, this may inform targeted, transdiagnostic treatments that may be effective in reducing both depression and anxiety complaints in affected patients.

Overall, although all of the aforementioned research approaches have different viewpoints on the pathophysiology of mental disorders and have several pros and cons, they all have the ultimate goal to improve diagnostics and to elucidate underlying pathophysiological mechanisms that can inform tailored treatments. It should not be pursued to select the best approach to study the close relationship between depression and anxiety, but this relationship should rather be studied from different perspectives or even integrate different approaches. For example, the network approach may benefit from including behavioral factors in the symptom network, so that these factors can be linked to specific psychiatric symptoms. Empirical insight can then be obtained from integrative different perspectives, which may ultimately provide a better understanding in the complexity of the pathophysiology of depression and anxiety and may contribute to the development of more effective tailored treatments. Thus, both the use of a hypothesis-driven perspective focusing on anxious distress in MDD as well as other innovative research approaches studying the mood/anxiety spectrum, other anxiety dimensions, or depressive symptom profiles other than the anxious depression profile, are worthwhile to examine and should be compared against each other.
Potential implications for clinical practice

In this thesis, the main objective was to establish whether anxious distress in depression is a distinct and clinically relevant symptom profile. This is important to investigate, as substantial evidence points out that patients with anxious depression generally have a poorer prognosis and are less responsive to treatment than those with non-anxious depression. However, various definitions for anxious depression have been used across previous studies and therefore results are not always directly comparable. The relatively new DSM-5 anxious distress specifier may potentially overcome this problem, as it may contribute to a more uniform, clinically relevant definition. Evidence on this specifier is, however, relatively limited and it should thus be further examined whether it can be used to identify more homogeneous subgroups of patients with MDD and anxiety that likely may benefit from specific treatment strategies. Furthermore, elucidating a distinct neurobiological profile of DSM-5 anxious distress in depression may not only help its recognition as a distinct symptom profile, but also identify potential neurobiological treatment targets that may ultimately improve treatment strategies.

Measurement-based care: is the DSM-5 anxious distress specifier a clinically useful tool?

The DSM-5 anxious distress specifier may have several important implications for clinical practice. First, the specifier may have particular value in primary care. Patients with MDD and anxiety features are common in primary care, and are now often unrecognized and underdiagnosed, or misdiagnosed as a residual diagnosis ‘not otherwise specified’. Also, time and expertise are generally limited in primary care and may often restrain thorough psychiatric assessment. Therefore, this short, simple, 5-item specifier is a quick and easy-to-use tool that may serve primary care clinicians who suspect a mental disorder in recognizing and identifying prominent anxiety features in patients with MDD. Second, the anxious distress specifier may be clinically useful not only in primary care, but also within psychiatric practices. This thesis showed that overlap between the anxious distress specifier with formally diagnosed comorbid anxiety disorders was poor (Cohen’s κ=0.09), which indicates that the specifier may capture patients with MDD suffering from prominent anxiety features predictive of poorer outcomes, who may not be fully captured within the DSM-based anxiety disorder diagnoses. Moreover, findings in this thesis show that the anxious distress specifier outperforms comorbid DSM-based anxiety disorder diagnoses as a longitudinal predictor for important clinical outcomes in patients with MDD. Additionally, the short 5-item specifier is much easier and less time-consuming than formally diagnosing a full-blown comorbid anxiety disorder diagnosis. So, when it comes to identifying those with a poor course, the specifier may be an easier method than formally diagnosing full-blown anxiety diagnoses, without suggesting that these anxiety
features may include, replace or differentiate anxiety disorders (which is also reflected in the Cohen’s $\kappa$ of 0.09). Indeed, measurement of a full-blown comorbid anxiety disorder in patients with MDD is still legitimate, because it could provide other clinically relevant information for instance regarding specific treatments. However, it may be worthwhile to measure anxious distress in patients with MDD as a first step because it is a rather generic anxiety marker that can quickly identify depressed patients with a poor course who may not be fully captured within comorbid anxiety disorder. Though effective novel therapeutics are not yet available for patients with MDD plus anxious distress, the specifier can certainly be used by clinicians for proper treatment monitoring and planning in these patients when conventional treatment strategies are applied. When clinicians initiated conventional treatment strategies in patients with MDD who meet the anxious distress specifier, treatment effectiveness by means of a decrease in anxious distress symptoms can be more frequently measured by the short 5-item specifier and treatment strategies can be modified or intensified if necessary, depending on the obtained information from these monitoring assessments. Based on this first step, clinicians can decide whether further measurement of potential anxiety symptoms—other than those of the anxious distress specifier—and diagnosing specific types of anxiety disorders is needed, as not all anxiety symptoms qualify for patients with the specifier. Third, the findings presented in this thesis showed that the DSM-5 anxious distress specifier is longitudinally predictive of differential treatment response, greater depression chronicity and severity over time, and greater frequency of side effects in patients with MDD on recently initiated antidepressant treatment. This suggest that the DSM-5 anxious distress specifier picks up an important group of depressed patients with anxiety features that likely may benefit from specific, novel, or more intensive treatment strategies. The Dutch multidisciplinary guideline for depression provides guidelines for stepped-care by structured treatment algorithms for patients with depression [51]. In addition to basic interventions (e.g. psychoeducation and day structuring) and first-step interventions (e.g. self-management and running therapy), mild and moderate depressive episodes are usually treated by either psychotherapy or an antidepressant whereas more severe depressive episodes are usually treated with combination therapy of psychotherapy and antidepressants. If depressed patients with significant anxiety features yield inadequate response to an antidepressant or psychotherapy after a sufficient duration, switching antidepressants or intensifying treatment strategies should be considered. These strategies may include combination therapy of antidepressants and psychotherapy or augmentation with the anxiolytic drug buspirone or atypical antipsychotics. Also, novel pharmacological treatments may have specific efficacy for patients with depression and anxiety. This thesis showed that the DSM-5 anxious distress specifier exhibited one of the strongest associations with LPS-stimulated inflammation index score which suggests that these 5 simple criteria are enough to select a group of MDD patients who are more homogeneous biologically than
the MDD population as a whole. Since it has been shown that several anti-inflammatory treatments such as TNF-α antagonists \[63\], nonsteroidal anti-inflammatory drugs and cyclooxygenase-2 inhibitors \[64\] may improve depressive symptoms, the anxious MDD subgroup may specifically benefit from anti-inflammatory agents or new antidepressants with a target on inflammation. However, these results are preliminary and should be first further investigated in advance of clinical adoption of these treatments in patients with MDD and anxiety.

Furthermore, when treating patients with MDD and significant anxiety features some important treatment aspects should be taken into account. Research showed that patients with anxious depression are likely to suffer more from antidepressant side effects than those without anxious depression. This thesis showed that this also specifically accounts for depressed patients with anxious distress. Greater frequency of antidepressant side effects is often problematic because it may lead to early discontinuation of treatments. Close monitoring of pharmacological tolerability by means of side effects and possibly starting a lower dose with upward titration may therefore be important particularly in this group of patients. Clinicians should further be aware of properly weighing the advantages and disadvantages of pharmacological treatments in these patients. Thus, the anxious distress specifier may also be clinically useful for proper treatment planning and monitoring in patients with MDD and anxious distress.

Finally, in this thesis the role of anxious distress and other depression characteristics on somatic health consequences was explored in patients with MDD. We first found that persons with current MDD are at risk of excess somatic diseases as compared to controls, which was not restricted to one single somatic disease category. This suggests that the association of current depression with incidence of somatic diseases is rather generic and may not be associated with specific underlying neurobiological mechanisms in specific groups of depressed patients. Prevention strategies may therefore be much simpler, for instance, early interventions such as preventive exercise group programs can be applied to many depressed patients simultaneously. Of the clinical depression characteristics, the depressive subtypes did not substantially differ in their somatic disease incidence risks. However, higher depression symptom severity showed a dose-response relationship with the risk of somatic disease incidence, and also mood depressive symptoms and somatic/vegetative depressive symptom dimensions were associated with increased somatic disease incidence. These results provide important insight into the impact of specific clinical depression characteristics on 6-year incidence of common somatic diseases. This may hold important clinical implications in that it may inform more specific prevention and treatment strategies in depressed patients. Patients with current MDD, specifically those with high depression severity, and mood and somatic/vegetative symptoms should be
monitored more closely and routinely screened on risk factors for somatic disease in order to reduce incidence of somatic diseases.

Thus, based on the findings on the DSM-5 anxious distress specifier so far, it seems that this specifier is an easy and clinically useful tool to systematically measure and monitor anxiety features on a routinely basis in patients with MDD which in turn can be used for proper treatment planning.
**Recommendations for future research**

The findings presented in this thesis collectively suggest that anxious distress in MDD is a distinct and clinically relevant symptom profile of depression. Nonetheless, the DSM-5 anxious distress specifier should be further investigated and compared with other anxiety constructs. Here, we provide several directions for future research based on the studies that were carried out in this thesis.

In Chapter 2 and 3, it was demonstrated that the anxious distress specifier captures a clinically valid and reliable construct predictive of poor clinical and treatment outcomes. The anxious distress specifier was predictive of lower MDD remission rates, higher depression severity after 1 year and 2 years, and greater frequency of side effects in patients with new-onset MDD who started adequate antidepressant treatment, although these findings were based on observational data from a naturalistic cohort study. Ideally, a placebo-controlled RCT design is required in order to adequately examine treatment response and remission. Since the introduction of the DSM-5 anxious distress specifier, thus far, no RCTs have been conducted to study treatment outcomes in patients with depression and anxious distress. Previous studies that were conducted before the introduction of the DSM-5 anxious distress specifier did examine treatment outcomes in patients with anxious depression but defined this by other anxiety scales [65]. Future research should thus examine the anxious distress specifier’s usefulness to stratify patients and examine treatment outcomes in placebo-controlled RCTs. Also, some research efforts have been made to establish effective novel pharmacological treatments for patients with MDD and anxious depression [66], but have not been conclusive because of the insufficient number of studies (Chapter 5). Up until now, only one study examined a novel therapeutic agent specifically in patients with MDD with and without anxious distress [67]. It was found that adjunctive brexipiprazole was effective in both MDD patients with and without anxious distress [67]. Clearly, more research is warranted to identify novel pharmacological treatments that are safe and effective for patients with MDD and anxious distress. With regard to psychotherapy, patients with depression and a comorbid anxiety disorder are likely to be patients requiring a combination of antidepressant medication and psychotherapy [68]—where psychotherapy may need to focus not only on depression, but also on anxiety and avoidance behaviors—, this treatment combination may also be effective for patients with MDD and anxious distress. However, to date, no other studies have been conducted on the effectiveness and place of psychotherapy in the treatment plan of patients with MDD specifically meeting criteria of the anxious distress specifier. It is interesting to examine this in order to further optimize treatment strategies for these patients.
Chapter 4 showed novel neurobiological insight into anxious distress in MDD in light of inflammation. It was found that anxious distress and related dimensional anxiety features were associated with increased immune dysregulation in patients with MDD, which may be responsible for the poor clinical and treatment outcomes observed in these patients. However, no other studies have been carried out that directly examined inflammation in MDD and anxiety and more research on this is required. Also, this study used cross-sectional data and therefore no causal inferences can be made. In order to determine causality directions in the link of inflammation and anxious distress in MDD, future research should further investigate the association between immune dysregulations and MDD with anxious distress using longitudinal data. In addition to the immune system, future research is warranted to uncover other biological correlates in patients with MDD and anxious distress such as the hypothalamic-pituitary-adrenal (HPA)-axis and autonomic nervous system. Also, it was shown in Chapter 5 that anxious depression is associated with increased cortical thinning in the orbitofrontal and anterior cingulate cortex (ACC) and increased functional connectivity between limbic-striatal and prefrontal regions. As the ACC, insula and other regions structurally and functionally implicated in anxious depression also play a role in predicting depression course and treatment outcome [69-71], deficits observed in these regions may be linked to the poor clinical outcomes in anxious depression. Further research is warranted to replicate findings and to confirm distinct neurological correlates in depression with co-occurring anxiety. By elucidating and obtaining a better understanding in the neurobiological profile of anxious distress in MDD, this may aid not only its definition but also its recognition as a distinct symptom profile and ultimately its treatment by uncovering potential neurobiological targets.

Though various definitions of anxious depression including anxious distress in MDD were related to poor clinical outcomes and differential neurobiology as was shown in Chapter 5, it is important to identify which underlying dimensional anxiety features captured in different anxious depression definitions are driving associations with clinical outcomes and biology. In order to address this, innovative research approaches should be used that involve transdiagnostic studies that span across disorders on the full mood/anxiety spectrum within a common dimensional framework. Transdiagnostic approaches that might be used are, for example, the RDoC framework [52] and the network approach based on the use of a statistical network analysis technique [57]. Along these lines, it is important to not only further investigate the future recommendations on the DSM-5 anxious distress specifier suggested in this thesis, but also to investigate other dimensional anxiety constructs using more transdiagnostic approaches and compare them with the specifier.
The study described in Chapter 6 shows that patients with an active depressive episode are significantly at risk of excess somatic diseases, which is not restricted to one single somatic disease category. While these findings may hold important clinical implications, it is the first longitudinal study—to our knowledge—that examined whether 6-year incidence of a wide range of common somatic diseases differed between persons with current MDD, remitted MDD, and healthy controls (n=616), and that examined the role of clinical depression characteristics including depression severity, depressive symptom clusters, individual depressive symptoms and depressive subtypes. Therefore, more research is needed to replicate and confirm these findings. Also, a dose-response association was found between depression severity and higher somatic disease incidence and seemed to be mainly driven by depressive mood and somatic/vegetative symptoms. Future research should examine by which mechanisms somatic/vegetative and mood depressive symptoms may contribute to higher somatic disease onset risks. A better insight into these mechanisms may contribute to improvements in prevention strategies and potential treatment targets for the development of common somatic diseases in patients with MDD.
General conclusions

Throughout the history, multiple paradigm shifts have occurred within the field of Psychiatry. Nowadays, a plethora of research focuses on ‘precision medicine’ in Psychiatry, using innovative approaches to obtain a greater understanding in the pathophysiology of psychiatric disorders. In doing so, it is aimed to uncover specific biomarkers that may predict specific treatment response and inform tailored treatment options in more homogeneous subgroups of patients suffering from psychiatric disorders [72]. Within depression research it has been increasingly recognized that heterogeneity of the MDD diagnosis is hindering research, and major neurobiological or genetic scientific breakthroughs have not yet been reached. As a result, focus in depression research shifted towards identifying more homogeneous depression subgroups that may have a differential underlying neurobiological basis so that effective novel treatment interventions can be developed.

This thesis aimed to establish whether anxious distress in MDD is one of such a more homogeneous and clinically important symptom profile of MDD. This objective was addressed from different perspectives: the longitudinal predictive validity and the role of anxious distress was examined in clinical course trajectories, treatment outcomes, somatic health consequences and touches upon its potential underlying neurobiology in light of the immune system in patients with current MDD. Also, the value and place of anxious distress in depression within the research field of anxious depression is explored. The results presented in this thesis collectively showed that the DSM-5 anxious distress specifier is a clinically useful construct to identify patients with MDD and prominent anxious symptoms predictive of a poorer course and treatment outcomes than those without these anxious features. This makes the simple 5-item anxious distress specifier a quick and easy-to-use indicator to select subgroups of patients with anxious depression in need of novel or more intensive treatment and follow-up, which can be used both in primary care and psychiatric practices. Also, anxious distress in MDD seems associated with increased immune dysregulation as patients with MDD and anxious distress had higher innate cytokine production capacity versus those without anxious distress. Furthermore, the anxious distress specifier appears to be a welcome development within the field of anxious depression, although evidence on the specifier is relatively limited to draw firm conclusions about its utility as the ‘gold standard’ to measure and define concurrent anxiety in depression.

Overall, this thesis contributes to a better insight into the clinical value of anxious distress in MDD by showing that it is a common and distinct depressive symptom profile associated with a poorer course, lower treatment response and increased immune dysregulation, compared to ‘pure’ depression. Therefore, this work moves the research field
of anxious depression forward, in that it provides important evidence on the place and value of the anxious distress specifier to use it as an anxious depression measure. Furthermore, this thesis unraveled a piece of the heterogeneity of MDD. Nevertheless, more research is warranted into the DSM-5 anxious distress specifier, not only to replicate our findings but also to compare the specifier with other dimensional anxiety constructs. More generally, research efforts should be undertaken to identify which underlying anxiety features captured in different anxious depression definitions are driving associations with clinical outcomes and neurobiology. For this, novel transdiagnostic approaches may be useful such as the RDoC framework [52] or the network approach [57] [73]. In doing so, this hopefully will elucidate the pathophysiology of disorders that span across the mood/anxiety spectrum, which will inform specific treatment interventions and ultimately will diminish the great burden of patients affected with MDD as well as the major public health consequences of MDD.
References


Das-Munshi J, Goldberg D, Bebbington PE, Bhugra DK, Brugha TS, Dewey ME, Jenkins R, Stewart R,


