SUMMARY AND GENERAL DISCUSSION

The overarching aim of this thesis was to gain insight into the measurement of clinical progression in Alzheimer’s disease (AD). We described the development and validation of the Cognitive-Functional Composite (CFC) as a novel measure for clinical progression in MCI and mild dementia. Next to that, we investigated whether existing cognitive outcomes differ in their sensitivity to decline at different clinical stages within the AD spectrum. The main findings of this thesis are:

1. The CFC is a concise measure covering relevant aspects of cognition and function.
2. The CFC has good test-retest reliability and feasibility of use.
3. The CFC score is related to other clinical and biological measures of disease severity.
4. The CFC is sensitive to clinical progression over a period of one year.
5. The CFC provides a more refined measure of clinical progression as compared to traditional clinical endpoints.
6. Individual cognitive tests vary in their sensitivity to decline at different stages of AD.

In this chapter, we summarize the findings of this thesis in more detail, discuss our results as well as methodological considerations, and present the conclusions of this thesis. We conclude with the clinical implications of our findings and directions for future research.

Summary

Novel approaches to assess clinical symptoms of Alzheimer’s disease

Chapter 2.1 provides the rationale for the Catch-Cog study of the novel CFC. We summarize the limitations of traditional cognitive and functional tests to measure progression in MCI and mild dementia, which leads to the need for a brief, reliable, valid and sensitive measure capable of capturing clinically meaningful cognitive decline. We describe the design of our proposed composite measure combining cognition and function, which is based on preparatory work on the Cognitive Composite and Amsterdam IADL Questionnaire, and we summarize existing evidence on the reliability and validity of those separate measures. Subsequently, we provide an overview of the Catch-Cog study design that includes an independent longitudinal construct validation of the novel CFC. We describe our hypotheses that the CFC would be able to capture progression
in MCI and mild dementia, and that the CFC would be related to other reference measures of disease progression. We conclude with our expectations that the Catch-Cog study of the CFC could contribute to improved monitoring of disease progression and more effective treatment evaluation in the MCI and mild dementia stages of AD.

Chapter 2.2 describes the development and validation of a shortened version of the Amsterdam IADL Questionnaire, which we aimed to incorporate in the CFC. Based on an iterative process combining input from experts, missing data and item response theory (IRT) analyses, we designed a short version containing 30 items. We thereby reduced its administration time by approximately 10 minutes. We showed that, although significantly shorter, the short version has maintained the psychometric qualities of the original version. We found that short version scores are in high concordance with the original version, and that the short version has similar internal consistency as the original version. We also demonstrated its measurement precision along the entire spectrum of IADL functioning. Furthermore, expert ratings confirmed that the selected items are relevant and that all key concepts of IADL functioning are included. We found that short version scores are in high concordance with other cognitive and functional measures, and that scores worsen on the clinical spectrum from normal cognition to dementia. Altogether, these results illustrate that the short version of the Amsterdam IADL Questionnaire yields a concise and user-friendly tool to efficiently measure functional impairment in predementia and early dementia stages.

Psychometric evaluation of the cognitive-functional composite

Chapter 3.1 describes the first validation step of the novel CFC. We investigated the stability of the CFC by performing a test-retest study with 2-3 weeks between assessments, in a group of MCI and mild dementia patients and cognitively healthy participants. We also evaluated feasibility of the CFC, with a focus on patients’ experiences with respect to its administration time, modality and perceived burden. Our findings show that all CFC components have moderate to high test-retest reliability, and that the overall CFC score provides a stable measure of cognition and function. Lastly, qualitative results of patient interviews indicate the CFC’s good feasibility of use. Patients mentioned that they did not perceive the test administration as burdensome, and the total duration (20-25 minutes) was experienced as acceptable. In addition, test materials were described as ‘clear’, and ‘very readable’. Taken together, this study demonstrates the reliability of the CFC, but also underlines the feasibility and comprehensibility of the cognitive component of the CFC.

In chapter 3.2, we investigated the relationship between cortical atrophy and
functional impairment as measured with the Amsterdam IADL Questionnaire. We found that worse Amsterdam IADL scores are related to less cortical volume across the clinical spectrum ranging from SCD to dementia. We demonstrated that this relationship is independent of age, sex, education and markers for vascular injury. VBM indicated that associations between IADL and grey matter volume are mostly specific for typical AD brain regions, such as the medial temporal lobes including the hippocampi, and the cingulate cortex and associated areas including the precuneus. When restricting the sample to participants who are biomarker positive for AD, we again found that IADL functioning is related to grey matter volume across the AD spectrum profoundly in the left medial-temporal lobes and precuneus. These findings illustrate that the Amsterdam IADL Questionnaire is able to detect problems in complex activities of daily living that are associated with AD specific neurodegeneration.

In chapter 3.3 we report on the second validation step of the CFC. We performed a cross-sectional study using the baseline data of the Catch-Cog cohort, in order to evaluate several quality aspects of the novel CFC. We examined the CFC’s construct validity, clinical relevance and suitability for the target population in comparison to traditional tests of cognition and function. Factor analyses confirmed the underlying structure of the CFC, reflecting the domains memory, executive functioning (EF) and IADL functioning. We also found that CFC scores decrease across the clinical spectrum from SCD to dementia, as in line with the clinical manifestations of those groups. Moreover, we demonstrated that worse CFC performance is associated with greater cognitive decline as reported by the informant, poorer quality of life, higher caregiver burden, more apathy and less cortical volume. We also showed that in our sample of MCI and mild AD dementia patients, CFC scores yield fewer range restrictions in scoring as compared to traditional tests of cognition and function, indicating improved suitability for the target population. In summary, these findings indicate that the CFC has good construct validity, captures clinically relevant aspects of disease severity and provides a more useful outcome measure than traditional tests to evaluate cognition and function in MCI and mild AD dementia.

**Measuring clinical changes over time**

In chapter 4.1 we investigated the CFC’s sensitivity to change over time in comparison to traditional tests using longitudinal data of the Catch-Cog cohort. Overall, we found that the CFC captures clinically meaningful decline over one year, and that the CFC components exhibited greater change as compared to traditional clinical endpoints. In MCI, the functional component of the CFC detected decline after one year, whereas traditional measures failed to do so. In mild AD dementia, the CFC detected change as early as 6 months after baseline.
and exhibited greater change than the CDR-SB at all follow-up time points. Finally, we demonstrated that annual change on the CFC is associated with decline in cognitive and functional abilities as reported by the study partner. Based on these findings, we can conclude that the CFC is capable of detecting clinical meaningful cognitive decline, and provides a superior alternative approach to the use of traditional clinical endpoints. The CFC is an improved method for monitoring clinical progression and could enhance the evaluation of clinical trials aimed at MCI and mild dementia stages of AD.

As AD research and clinical trials are moving towards earlier, asymptomatic stages of AD, the expected degree of observable cognitive decline within the course of a trial is reduced. However, it is unknown whether currently-used cognitive tests vary in their ability to capture decline at different clinical stages. In chapter 4.2 we therefore investigated the sensitivity to decline of several widely-applied cognitive tests at different clinical stages as outlined in the NIA-AA research framework [1]. We used longitudinal cognitive data from AD biomarker positive participants whose clinical status ranged from cognitively normal to severely impaired, originating from four well-defined study cohorts. We operationalized the NIA-AA clinical scheme into measurable criteria, which enabled classifying the majority of those participants into the four defined NIA-AA stages. We found that cognitive outcomes differ in their sensitivity to decline by clinical stages on the AD continuum, indicating that stage-specific composites are needed to effectively evaluate (early) AD trials. We showed that with increasing clinical stage, optimal cognitive outcomes cover broader cognitive domains. These results can provide guidance on the selection of cognitive endpoints, particularly when the NIA-AA 2018 framework is applied to define the treatment population.

**General discussion**

**Validation of a novel outcome measure**

The first goal of this thesis was to investigate whether the CFC could provide a suitable measure for clinical progression in MCI and mild dementia. To achieve this, the different chapters in this thesis addressed several key properties for health-related outcome measures, such as content validity, reliability, construct validity and responsiveness [2].

Content validity can be defined as the degree to which the content of an instrument is an 'adequate reflection of the construct to be measured' [3]. It is usually considered the most important measurement property, as it should be evident that 1) all the items of a measurement instrument are relevant for measuring the construct of interest (relevance); 2) all key concepts are included...
(comprehensiveness); and 3) all items, response options, and instructions are understood by the target population as intended (comprehensibility) [3]. We addressed these three aspects by involving experts in the development of the CFC, and by reviewing empirical evidence on the different components included in the CFC. For the cognitive component, we selected existing measures covering the cognitive domains that are considered relevant for measuring progression in incipient dementia due to AD, according to experts in the field (chapter 2.1). An advantage of this approach is that the content validity of those individual tests has already been demonstrated previously [4]. We also showed that the items of the functional component of the CFC were considered relevant by dementia researchers and clinicians (chapter 2.2). Additionally, quantitative analyses indicated that the functional component covered the entire spectrum of IADL functioning, and could thereby considered comprehensive (chapter 2.2). Good comprehensibility for both CFC components was confirmed by interviewing patients and caregivers on their experiences regarding the content and administration of both the functional component (chapter 2.2) and cognitive component (chapter 3.1).

A second important element of a measurement instrument relates to its stability, to ensure that it provides a similar score when no changes have occurred in the construct of interest [3]. Our test-retest study showed that the CFC provided a stable score after 2-3 weeks follow-up (chapter 3.1), which is a timeframe in which we do not expect people with MCI or dementia to change regarding their cognitive functioning. Third, assessing the construct validity of a measurement instrument is important, as this provides supporting evidence that it measures the construct that it purports to measure [3]. Good construct validity for the CFC was confirmed based on the associations we found with other clinical and biological measures of disease severity (chapter 3.2 and chapter 3.3), as well as by differences in CFC scores between clinical groups (chapter 3.3). Finally, responsiveness of a measurement instrument refers to its capability to detect change in the construct to be measured, which is of particular importance for an outcome measure that is intended to assess change over time. Evidence for the responsiveness of the CFC was provided in chapter 4.1, in which we showed that CFC scores declined over one year.

To summarize, Figure 1 provides an overview of how the different chapters of this thesis contribute to the converging evidence that the CFC is a suitable measure for clinical progression in MCI and mild dementia.

**Optimizing cognitive outcomes for different clinical stages of AD**

Our second goal was to investigate whether cognitive outcomes vary in their sensitivity to decline at different clinical stages within the AD spectrum. This

*Summary and general discussion 195*
was addressed in chapter 4, with chapter 4.1 revealing differences in change on the CFC measures amongst SCD, MCI and dementia, and chapter 4.2 showing that individual cognitive tests vary in their sensitivity to change at different AD clinical stages as outlined in the NIA-AA research framework. Table 1 shows how the NIA-AA clinical staging and clinical syndrome staging schemes could be aligned, and which individual CFC cognitive tests were identified as sensitive in chapter 4.2. Although it should be noted the study in chapter 4.2 differed regarding study-sample and follow-up timeframe, the findings regarding the sensitivity of the CFC cognitive subtests were in agreement with the results obtained in chapter 4.1. Both studies suggest that the cognitive component of the CFC in its current form is 1) limited sensitive to changes in SCD (Stage 2); 2) partly sensitive to change in MCI (Stage 3), and; 3) evidently sensitive to change in dementia (Stage 4). Moreover, the finding that the majority of CFC cognitive tests were identified as sensitive in a large group of AD biomarker positive individuals at clinical Stages 3 and 4 (chapter 4.2), supports the use of the CFC in our target population of individuals with MCI and mild dementia due to AD. However, these results also imply that the CFC could potentially be further refined to improve its sensitivity to change in Stage 3, as some of the individual CFC cognitive tests did not seem to capture decline after one year in this stage.

Table 1. Sensitive CFC measures identified in chapter 4.2.

<table>
<thead>
<tr>
<th>Clinical status</th>
<th>NIA-AA staging scheme</th>
<th>Measures included in the CFC</th>
</tr>
</thead>
<tbody>
<tr>
<td>SCD</td>
<td>Stage 2</td>
<td>Category Fluency, Word Recall</td>
</tr>
<tr>
<td>MCI</td>
<td>Stage 3</td>
<td>Category Fluency, Digit Span, Word Recognition, Word Recall</td>
</tr>
<tr>
<td>Dementia</td>
<td>Stage 4</td>
<td>Category Fluency, Digit Span, Word Recognition, Word Recall, COWAT, Symbol Substitution</td>
</tr>
</tbody>
</table>
Combining cognitive tests with a functional measure: the bridge to clinical meaningfulness?

A recurring finding in this thesis was the added clinical value of the Amsterdam IADL Questionnaire as functional component of the CFC. For example, we found that associations between the CFC score and other measures of disease severity were mostly driven by the Amsterdam IADL score (chapter 3.3). Additionally, we showed that Amsterdam IADL Questionnaire is able to detect subtle impairment in individuals with SCD (chapter 2.1), and to capture decline over one year in MCI, whereas the cognitive component of the CFC failed to do so (chapter 4.1). These findings seem counterintuitive, as the assumed clinical trajectory of AD entails that cognitive impairment induces and thereby precedes functional impairment [7]. However, our findings do not argue against this conceptual understanding of cognitive impairment preceding functional change, but rather imply that existing paper-and-pencil cognitive tests may not provide the right tools to capture subtle cognitive decline. This is in line with previous studies that pointed towards the limited sensitivity of existing cognitive tests in early clinical stages of AD [8, 9]. A functional measure on the other hand, may be capable of capturing meaningful decline as reflected by increasing difficulties in complex activities of daily living. Previous studies on the Amsterdam IADL Questionnaire already demonstrated its added diagnostic and prognostic value to neuropsychological tests in dementia [5, 6]. The current thesis provides further evidence on the importance of the inclusion of a sensitive IADL measure to capture clinically cognitive decline in predementia stages of AD. More specifically, our results imply that the Amsterdam IADL Questionnaire could provide the missing link between cognitive test scores and clinical meaningfulness.

Worldwide, researchers and regulatory agencies have expressed the need for novel, sensitive tools that capture clinically meaningful decline in predementia stages of AD [10, 11]. This is of particular relevance in the context of clinical trials, since evidence of efficacy on a clinical meaningful measure is required to achieve regulatory approval of novel therapeutic interventions [12]. In an attempt to develop novel measures and methods that yield components of both functional and cognitive skills, several other endeavors have been undertaken. Examples include the Clinical Dementia Rating scale (CDR-SB) [13] and the recently designed Alzheimer's Disease Composite Score (ADCOMS) [14]. However, the clinical meaningfulness of those measures is yet uncertain, especially for the ADCOMS procedure which has been largely statistically-driven [15]. Furthermore, we showed that both the CDR-SB and ADCOMS are prone to ceiling effects in MCI and mild dementia as compared to the CFC (chapter 3.3). We also showed that the CFC could provide a more refined and meaningful measure of clinical progression (chapter 4.1), and thereby offers advantages over the use
of the CDR-SB as a measure of efficacy to effectively evaluate novel treatments targeting early symptomatic stages of AD.

Methodological considerations
There are several methodological considerations that should be considered when interpreting the findings of this thesis.

Cohorts. Different cohorts were used in the studies included in this thesis. Studies on the CFC were performed using data from the Catch-Cog cohort, which is an international, observational cohort enrolling individuals with SCD, MCI, AD dementia and DLB. The prospective character of this cohort enabled us to perform an independent validation of the CFC, which is a unique aspect of this study. However, limitations of this cohort include possible heterogeneity due to differences in recruitment strategies across study centers (clinically-based versus community-based), and the fact that not all participants had biomarkers available to confirm neurodegeneration. Furthermore, the relatively small sample-sizes of the SCD and DLB groups limited the interpretation of our findings in these groups. As such, our investigation of the CFC in SCD and DLB remained of rather explorative nature.

Other studies in this thesis were conducted using data from the Amsterdam Dementia Cohort (ADC), which is an observational memory-clinic cohort of the Alzheimer Center Amsterdam [16]. A major strength of this cohort is its extensive phenotyping. However, it should be noted that the Alzheimer Center Amsterdam is a tertiary memory-clinic specialized in young-onset dementia, resulting in a relatively young cohort that is less generalizable to older populations. In our last chapter, we combined data from the ADC with data from three North-American cohorts, including the community-based observational Harvard Aging Brain Study (HABS) cohort [17], the Alzheimer's Disease Neuroimaging Initiative (ADNI) [18] and National Alzheimer's' Coordinating Center (NACC) [19] research cohorts. Combining these four well-defined cohorts provided a large and unique sample of AD biomarker positive individuals covering the entire clinical spectrum of AD. However, pooling data across these studies had some limitations due to differences across cohorts regarding 1) cognitive tests that had been assessed; 2) time-intervals between follow-up visits; and 3) assessment methods to determine amyloid positivity. Furthermore, a general limitation of these observational cohorts includes the loss to follow-up, which can be regarded as non-random in that more severely affected individuals are probably earlier lost to follow-up [20]. This could have induced a selection-bias that may have affected the internal validity of our results.
Study design. The longitudinal study design of the Catch-Cog study including assessments at baseline, 3, 6 and 12 months may have influenced our results regarding the CFC's sensitivity to change over time. First, participants were only followed for one year, and it could be argued that this is rather short to observe evident decline in individuals with MCI or mild AD. However, both the A-IADL-Q and subtests of the CC were shown to be able to capture changes within the one-year timeframe [21, 22], so therefore we expected that one year would be sufficient to detect decline. Second, the time intervals between follow-up visits were relatively short, which may have led to practice effects on cognitive tests, particularly at 3 and 6-months follow-up visits. Practice effects reflect improvements in cognitive test performance that result from repeated exposure to the test [23]. They are a potential threat for longitudinal cognitive assessment, as they can underestimate true cognitive decline [24]. Previous studies on practice effects in the early stages of dementia are contradictory [25], but some of them have shown that practice effects on memory measures can be observed in people with MCI [26]. The results presented in this thesis are contradictory as well, as we found negligible practice effects in our test-retest study, while in our longitudinal study an improvement was observed in CFC cognitive test performance in SCD and MCI participants. Interestingly, this improvement was observed while alternate versions of word lists were used at each follow-up visit, implying that not the actual test material but rather the ‘familiarity of being tested’ may have induced practice effects [27]. More specifically, this could be attributed to reductions in anxiety, as levels of anxiety are often higher during first assessment and thereby negatively affect cognitive performance [28]. This further highlights that the concept of practice effects is rather complex, and that variables that impact these effects are not fully understood yet. As a result, it remains difficult to distinguish whether ‘no change’ in cognitive performance in our MCI cohort reflect absence of progression, or whether decline caused by progression was diminished due to factors such as practice effects.

Construct validation approach. A main challenge for the validation of the CFC was the absence of a gold standard for ‘clinical progression’. We aimed to obviate this with a construct validation approach, by including other clinical and biological measures as reference measures of disease severity. We included other cognitive and functional tests as reference measures of progression, however, a potential limitation of those tests was their expected limited sensitivity to change in our target population [29, 30]. Therefore, we also selected measures that would be less likely to suffer from range restrictions in scoring, such as informant-reports of cognitive decline, quality of life measures, and global cortical volume [31-33]. Altogether, the associations between the CFC and
these ‘silver’ standards of clinical severity could provide converging evidence for the clinical relevance of the CFC.

Furthermore, the inclusion of the traditional tests also enabled us to perform a direct head-to-head comparison between the CFC and traditional tests, which is a unique aspect of our study. In fact, we did not only perform an independent validation of the CFC, but of the traditional measures as well. This led to further evidence for the quality limitations of those traditional measures when assessing clinically meaningful cognitive decline in MCI and early dementia.

Conclusion

By investigating how the measurement of clinical progression in early AD could be refined, this thesis provided several insights on the progression of clinical symptoms due to AD in general. First, our findings further support that cognitive decline due to AD starts years before the onset of dementia, and that decline in preclinical and prodromal stages of AD could be captured by certain, but not all, neuropsychological tests. Moreover, our results highlight that clinical endpoints to assess change should be tailored to a specific clinical stage, in order to effectively capture cognitive decline in that stage. Our findings further imply that longer time intervals and tests addressing specific cognitive domains are needed to capture change at earlier clinical stages, that AD-related cognitive decline exponentially increases over the years, and that broader cognitive functions become increasingly affected. Finally, this thesis demonstrates the added value of measuring IADL functioning when assessing clinical progression in early stages of AD, implying that the utility of an everyday functioning measure goes beyond just its use for diagnostic purposes in dementia. We conclude that combining measures of cognitive and function is recommended to capture clinically relevant cognitive decline due to AD, even in clinical stages that occur before the onset of dementia.

Clinical implications

Although it was our main focus to improve the measurement of progression in the context of research and clinical trials, the results presented in this thesis also imply the utility of the CFC for use in clinical practice. We showed that the CFC yields a concise measure that could be used to monitor disease progression after a diagnosis of MCI or dementia has been established. In many clinical settings such as memory clinics, it is not always feasible to perform a complete neuropsychological examination at each follow-up consult, due to time or financial constraints. Furthermore, patients often experience the examination as burdensome, which may cause them to abort the testing procedure [34].

Summary and general discussion
However, a quantified measure of cognition and function may assist the clinician in determining whether there has been progression compared to a previous visit. The CFC provides an efficient measure for that purpose, as the cognitive tests can be administered within half an hour. In the meantime, the Amsterdam IADL Questionnaire can be independently completed by an informant. It should, however, be noted that the CFC is partly based on the ADAS-Cog, which is not commonly used in clinical practice. As we did not compare the CFC with a full neuropsychological test battery, we cannot say whether the CFC outperforms a complete neuropsychological examination. However, results in chapter 4.2 suggest that far not all neuropsychological tests are sensitive to decline in early clinical stages of AD. We think that the CFC is less time-consuming and thereby probably less burdensome as compared to a full neuropsychological test battery, while sufficiently comprehensive enough to capture clinically relevant change over time. Additionally, our results imply that the addition of the Amsterdam IADL Questionnaire could enhance the ecological validity of the neuropsychological tests scores that are generally used in clinical practice.

**Future perspectives**

As AD clinical trials are moving towards pre-dementia stages [35], the sensitivity to change of the CFC in the MCI stage warrants further investigation, because the cognitive component did not detect decline in this stage over a period of one year. One approach to improve the sensitivity of the selected cognitive tests could be the use of IRT, which is the same scoring technique that is applied to the Amsterdam IADL Questionnaire. When applying IRT scoring, individual items are weighted based on their difficulty level, and this information is incorporated when calculating a total score based on an individual's responses [36]. For example, items that decline relatively early in the disease course require a higher level of the cognitive functioning to successfully endorse that item, and will thus be more heavily weighted for the total score. This may enhance the measurement precision of the cognitive component score, particularly at the early part of the clinical spectrum. Previous studies have indeed shown that IRT could improve the sensitivity to change of existing cognitive tests that were initially scored using classical test theory [37]. Furthermore, simulation studies have shown that IRT yields a better method to analyze repeatedly measured data as compared to sum-score based analyses, as sum-scores tend to overestimate within person variance and underestimate between person variance, which hampers the detection of change over time [38].

Whereas we found that the CFC score declined over time, we did not investigate whether this change was associated with biological changes related to progression. Therefore, it would be interesting to relate longitudinal

---

**Summary and general discussion**
change on the CFC to changes in biomarkers of AD neurodegeneration, such as hippocampal atrophy or tau pathology [32, 39, 40], to examine whether the CFC captures clinical progression due to AD specific processes. Next to that, it would be interesting to further validate the CFC as outcome measure for progression in DLB, which is the second common cause of neurodegenerative dementia [41]. Limited evidence is available on the clinical course of DLB so far, and proper methods to capture changes in clinical symptoms are still lacking [42]. The Catch-Cog study results suggest that the CFC could be of use to measures progression DLB, however, research including a larger sample-size would be needed to elucidate this.

Finally, the ultimate validation of the CFC would be to confirm its responsiveness to therapeutic intervention [43], which could, by definition, only be accomplished by implementing it in future clinical trials. A first step to achieve this is to convince regulatory agencies that the CFC is a suitable and feasible measure for use in clinical trials that aim to halt clinical progression in prodromal and mild AD, or the so-called Stage 3 and 4 patients [15]. In principle, both the FDA and European Medical Agency (EMA) encourage the use of composite endpoints to evaluate the efficacy of novel therapies and interventions aimed at those stages [12, 44]. However, they also stipulate that those composite measures should be 1) carefully designed, 2) validated in an independent prospective cohort-study, and 3) ‘bear some relevance to existing tools for which historical experience exists’. Most of these aspects have been addressed in the validation study of the CFC so far, which could advance the approval of the CFC as primary outcome measure of efficacy [45]. In this way, the CFC could contribute to the still ongoing quest for a disease-modifying therapy for AD.
References


4. Lezak, M.D., Neuropsychological assessment. 2004: Oxford University Press, USA.


Summary and general discussion 203


Summary and general discussion