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PRONOSTIC DU CHOLANGIOCARCINOME INTRAHÉPATIQUE RÉSÉQUÉ

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« Knowing when to operate and not to operate is just as important as knowing how to operate, and is a more difficult skill to acquire »

Henry Marsh, Do No Harm, 2014

RESUME

Introduction. Alors qu'elle constitue le seul traitement curatif du cholangiocarcinome intrahépatique (CCIH), la résection reste associée à un taux de récidive supérieur à 60% et un taux de survie réelle à 5 ans inférieur à 20%. Une estimation fiable du pronostic ainsi qu'une meilleure compréhension de la biologie tumorale est essentielle pour améliorer le pronostic.

Méthodes. A l'appui des données clinico-biologiques de deux larges cohortes de patients avec CCIH réséqué (MSKCC, n=189 et AFC, n=522), trois objectifs ont été explorés. Tout d'abord, définir quel modèle pronostique publié est le plus performant. Ensuite, définir la fiabilité de l'évaluation pronostique préopératoire à partir de, respectivement, l'imagerie, des microARN (miR) circulants diagnostiques et du profil génomique tumoral. Enfin, évaluer l'impact pronostique de la survenue d'événements périopératoires tels que transfusion et morbidité.

Résultats. Premièrement, les nomogrammes apportaient une meilleure estimation pronostique en comparaison à la classification AJCC 7ème édition. Deuxièmement, la taille et la multifocalité tumorale sur l'imagerie préopératoire permettaient de différencier deux groupes de patients de pronostic clairement distincts ($p<0,001$). L'existence d'une mutation d'un gène de remodelage de la chromatine (BAP1, ARID1A, PBRM1) tendait à être associé à une survie sans récidive plus favorable qu'en l'absence de mutation ($p=0,09$). Alors qu'ayant un potentiel comme marqueur diagnostique circulant, miR21 et miR221 n'étaient pas associé à la survie. Troisièmement, la transfusion peropératoire n'impactait pas la survie à long terme alors que la survenue d'une complication sévère (grade Dindo-Clavien > 2) était indépendamment associée à une survie globale plus courte ($p=0,002$).

Conclusion. Alors que les nomogrammes postopératoires apportent une meilleure estimation pronostique, le développement de modèles pronostiques préopératoires est faisable notamment à partir de l'imagerie et de marqueurs biologiques tumoraux complémentaires.

Mots-clés : cholangiocarcinome intrahépatique ; résection ; survie ; modèles pronostiques ; génomique ; microARN circulant

ABSTRACT

Introduction. Complete resection stands as the only curative option for intrahepatic cholangiocarcinoma (IHCC). Still, prognosis remains poor after resection due to a recurrence rate over 60% leading to actual 5-year survival rates below 20%. Reliable prognostic estimation and better understanding of tumor biology would be of interest for improving IHCC prognosis.

Methods. Using clinical and biological data from two large cohort of resected IHCC (MSKCC, n=189 and AFC, n=522), three objectives have been explored. First, assessing the performances of different published prognostic models. Second, defining the reliability of preoperative prognostic estimation using imaging, tumoral genomic profiling and circulating tumoral microRNA (miR). Third, evaluating the prognostic impact of perioperative events such as blood transfusion and morbidity.

Results. First, nomograms displayed better prognostic accuracy over the AJCC 7th edition staging system. Second, tumor size and multifocality on preoperative imaging allowed patient stratification in groups statistically different regarding prognosis ($p<0.001$). Further, the presence of chromatine remodeling gene mutations (BAP1, ARID1A, PBRM1) tended towards longer recurrence-free survical (p=0,09). Some diagnostic circulating miR such as miR21 and miR221 were not associated with survival. Third, in contrast with intraoperative transfusion, the occurrence of severe morbidity (Dindo-Clavien grade > 2) was independently associated with shorter overall survival (p=0.002).

Conclusion. Nomograms outperform conventional staging system. Preoperative prognostic estimation is feasible and reliable using imaging. Identifying new prognostic biomarkers would help refining preoperative prognostic estimation.

Key-words: intrahepatic cholangiocarcinoma; resection; survival; prognostic models ; genomic profiling; circulating microRNA

SOMMAIRE

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INTRODUCTION

Le cholangiocarcinome intrahépatique (CCIH) est une tumeur maligne développée à partir de l'épithélium biliaire intrahépatique, c'est-à-dire en amont des convergences biliaires droite et gauche (1). Il constitue la seconde cause de tumeur maligne primitive intrahépatique (10-15%) après le carcinome hépatocellulaire. De plus, bien que certaines données soient conflictuelles, la plupart des études épidémiologiques rapportent une augmentation de son incidence au cours des trois dernières décennies (2–4).

Alors que certains biomarqueurs à visée diagnostique émergent, le diagnostic est rarement précoce et la maladie est le plus souvent avancée et non résécable au moment du diagnostic (5). Dans cette situation, la survie médiane est classiquement limitée à moins de 18 mois sous chimiothérapie systémique conventionnelle (6,7). A l'inverse, lorsqu'une résection carcinologique complète associant hépatectomie et curage pédiculaire hépatique est réalisée, la survie globale médiane rapportée va jusqu'à 39 mois et le taux de survie globale actuarielle à 5 ans jusqu'à 39% avec cependant, un taux de récidive élevé, variant entre 62 et 79% et survenant le plus souvent au niveau intrahépatique (5,8,9). Toutefois, le taux de survie globale réelle à 5 ans semble plutôt varier entre 10% (données non publiées) et 22%, soulignant que la survie à long terme sans récidive est rare (10).

Ajoutée à la chirurgie, l'indication de la chimiothérapie adjuvante a longtemps été débattue puisque découlant de l'extrapolation de résultats chez des patients en situation palliative (11–13). Plus récemment, l'essai contrôlé randomisé français PRODIGE 12 a montré l'absence de bénéfice en termes de survie globale d'une chimiothérapie adjuvante associant gemcitabine et sels de platine après chirurgie pour cancer du tractus biliaire (14). Toutefois, plusieurs études ont rapporté l'intérêt d'une chimiothérapie adjuvante dans certains sous-groupes de patients avec des caractéristiques tumorales péjoratives telles qu'un envahissement ganglionnaire ou une résection R1 (15–17). Plus récemment, Primrose et al. ont rapporté les résultats de l'étude randomisée contrôlée BILCAP montrant le bénéfice de la capécitabine orale en adjuvant (18).

Les principaux facteurs pronostiques sont bien identifiés dans la littérature, avec notamment l'envahissement ganglionnaire (statut N), la radicalité de la résection (statut R), la taille et la multifocalité des lésions (statut T), ainsi que le type macroscopique (19–26). La plupart ont été inclus dans différents systèmes de classification et modèles pronostiques publiés (27–31). A l'heure actuelle, la classification de l'American Joint Committee on Cancer (AJCC) est la plus utilisée en pratique clinique. Néanmoins, les performances pronostiques de l'AJCC pour le CCIH ont été remises en question à plusieurs reprises (32–34).

HYPOTHESES

Alors que la résection chirurgicale reste la seule option curative, estimer le pronostic après résection reste primordial afin d'optimiser la séquence thérapeutique globale.

Tout d'abord, identifier les patients à haut risque de récidive après résection et leur proposer un traitement adjuvant représente l'approche idéale pour améliorer leur pronostic. Cette estimation repose classiquement sur des modèles combinant les facteurs pronostiques les plus pertinents. Dans le cadre du CCIH, la classification de l'American Joint Committee on Cancer (AJCC) est actuellement utilisée comme référence pour la stratification pronostique des patients. Cependant, comme préalablement rapporté pour d'autres tumeurs solides, les nomogrammes pourraient être plus performants que les modèles et classifications conventionnels (35,36). Dans le domaine du CCIH, deux nomogrammes ont été récemment publiés sans avoir été validés de manière externe, ni confrontés à la classification AJCC (28,29).

Plus pertinent encore, l'identification de ces patients à haut risque en préopératoire permettrait d'affiner la sélection des patients candidats à une chirurgie potentiellement lourde. En effet, d'après une récente revue de littérature, une hépatectomie pour CCIH est le plus souvent majeure (82%), nécessite fréquemment une résection/reconstruction biliaire ou vasculaire (23%), et est associée à des taux de mortalité et de morbidité atteignant 8% et 44% respectivement (37–39). Ainsi, identifier les patients à haut risque de récidive, qui pourraient donc ne pas bénéficier d'une résection, permettrait de leur éviter une résection potentiellement morbide à court terme et futile à long terme. De plus, cette identification préopératoire pourrait permettre d'orienter d'abord ces patients vers un traitement néoadjuvant, qui pourrait améliorer la sélection des patients et leur pronostic (40). Cependant, il n'existe actuellement pas de marqueur ou modèle pronostique préopératoire validé en pratique clinique courante. Alors que le dosage du CA19-9 sérique n'est actuellement recommandé que pour la surveillance après résection, la faisabilité de l'estimation pronostique après résection basée sur l'imagerie préopératoire a été peu rapportée. Certains biomarqueurs émergents tels que la génomique tumorale et les micro-ARN circulants (miR) sont accessibles en préopératoire et pourraient être utile à l'évaluation pronostique préopératoire. L'hétérogénéité tumorale du CCIH étant faible, une biopsie percutanée pourrait permettre d'établir de manière fiable le profil génomique tumoral pour identifier les altérations génomiques tumorales pronostiques mais aussi de potentielles cibles thérapeutiques (41). Les miR sont eux accessibles par simple prélèvement sanguin. Ils correspondent à de courts ARN non codants qui, à l'étape post-transcriptionnelle, sont capables de moduler l'expression d'oncogène et anti-oncogène intervenant ainsi dans la cholangiocarcinogénèse (42,43). L'expression de ces miR tumoraux disponibles à l'état circulant, peut ainsi être évalué sans avoir recours à une biopsie tumorale.

Enfin, certains événements péri-opératoires tels que le recours à la transfusion et la survenue de complications postopératoires sont des facteurs de mauvais pronostic décrits pour de nombreuses tumeurs solides mais dont l'impact pronostique n'a jamais été que peu exploré dans le domaine du CCIH (38,44). Ces événements périopératoires, s'ils ont un impact pronostique, pourraient avoir une place dans la discussion de l'indication d'un traitement adjuvant.

A partir de ces données, se posent trois hypothèses. La première pose la question de la performance de la classification AJCC et de sa position par rapport aux nomogrammes. La deuxième pose la question de l'évaluation pronostique préopératoire à partir de l'imagerie et de la place de la biologie tumorale tels que la génomique tumorale obtenue par biopsie ou les miR tumoraux circulants. La troisième pose la question du potentiel impact pronostique d'événements périopératoires tels que transfusion et complication.

OBJECTIFS

A partir de ces hypothèses, plusieurs problématiques pouvant améliorer le pronostic après résection du CCIH ont été identifié. Ces problématiques ont pu être explorées à travers les données de deux cohortes.

L'une correspondait à la base de données rétrospective des CCIH réséqués à visée curative au Memorial Sloan Kettering Cancer Center (MSKCC) de New York entre janvier 1993 et mai 2013. Les données disponibles de cette cohorte étaient cliniques et biologiques (plasma préopératoire, foie tumoral et non tumoral) et ont été obtenues après accord du comité d'éthique du MSKCC.

L'autre correspondait à la base de données rétrospective des CCIH réséqués à visée curative dans 24 centres en France entre janvier 1989 et mars 2009. Les données disponibles étaient purement cliniques et ont fait l'objet d'un rapport de l'Association Française de Chirurgie (AFC) en 2009.

Ces problématiques ont été rassemblées en trois objectifs détaillés ci-dessous :

1. Tout d'abord, différents modèles pronostiques disponibles, dont la classification AJCC, ont été évalués et confronté pour définir le plus performant.
2. Ensuite, le développement de marqueurs et modèles pronostiques préopératoires a été investigué. Un modèle pronostique préopératoire, basé sur l'imagerie, prédictif de récidive dans les 2 ans après résection a été développé et appliqué à une cohorte externe de validation. Deux potentiels biomarqueurs pronostiques disponibles en préopératoire ont également été explorés. L'un était l'analyse du profil génomique tumoral à partir de

biopise tumorale. L'autre correspondait à l'analyse des miRs tumoraux circulants obtenus par prise de sang en préopératoire.

3. Enfin, l'impact pronostique de la transfusion peropératoire et de la morbidité périopératoire ont été évalué.

EVALUATION DES MODELES PRONOSTIQUES EXISTANTS

EVALUATION DES MODELES PRONOSTIQUES EXISTANTS (Annexe 2) [45]

METHODES

Population d'étude

De janvier 1993 à mai 2013, 199 patients ont été consécutivement opérés d'un CCIH, à visée curative au Memorial Sloan Kettering Cancer Center (MSKCC). Tous les patients décédés dans les 90 jours postopératoires ou ayant bénéficié d'une résection palliative (résection R2, carcinose synchrone) étaient exclus de cette analyse. Cette cohorte incluait finalement 189 patients (Annexe 1).

Modèles pronostiques évalués

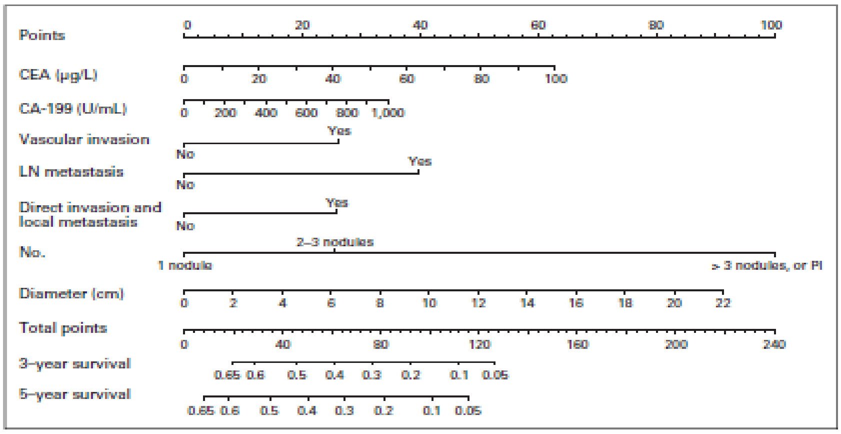
Trois modèles pronostiques ont été spécifiquement évalués. La 7ème édition de la classification AJCC qui prend uniquement en compte les données TNM de la tumeur réséquée (46). Le nomogramme de Wang et al, publié en 2013, inclut les données TNM et le taux sérique préopératoire d'ACE et CA19-9 (28). Il a été développé à partir d'une cohorte monocentrique rétrospective chinoise (n=367) et validée dans une cohorte issue du même centre (n=82). Le nomogramme de Hyder et al, publié en 2014, inclut l'âge du patient, les données TNM et la présence d'une cirrhose sous-jacente (29). Il a été développé à partir d'une cohorte multicentrique rétrospective internationale (n=514) et a été validé de manière interne, par technique de bootstrap.

Primary Tumor (T)	
TX	Primary tumor cannot be assessed
T0	No evidence of primary tumor
Tis	Carcinoma in situ (intraductal tumor)
T1	Solitary tumor without vascular invasion
T2a	Solitary tumor with vascular invasion
T2b	Multiple tumors, with or without vascular invasion
T3	Tumor perforating the visceral peritoneum or involving the local extrahepatic structures by direct invasion
T4	Tumor with periductal invasion
Regional Lymph Nodes (N)	
NX	Regional lymph nodes cannot be assessed
N0	No regional lymph node metastasis
N1	Regional lymph node metastasis present
Distant Metastasis (M)	
M0	No distant metastasis
M1	Distant metastasis present

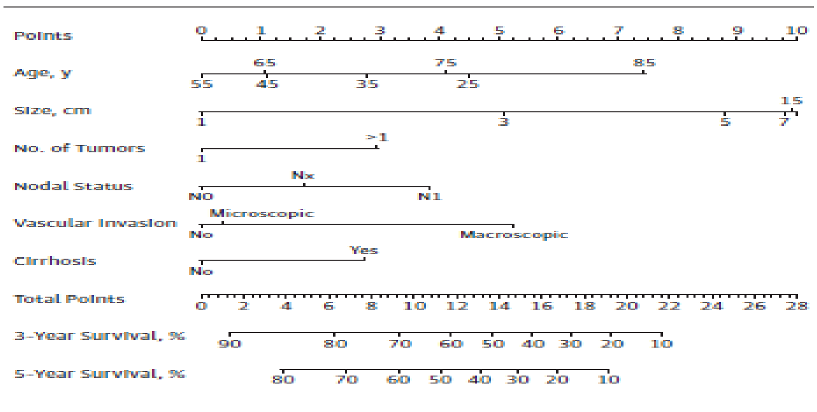
Anatomic Stage/Prognostic Groups

STAGE			
Stage 0	Tis	N0	M0
Stage I	T1	N0	M0
Stage II	T2	N0	M0
Stage III	T3	N0	M0
Stage IVA	T4 Any T	N0 N1	M0 M0
Stage IVB	Any T	Any N	M1

A. From Greene FL, Trotti A III, Fritz AG, et al. Liver. In: Edge SB, Byrd DR, Compton CC, et al, eds. AJCC Cancer Staging Manual. 7th ed. New York: Springer; 2010.



B.



C.

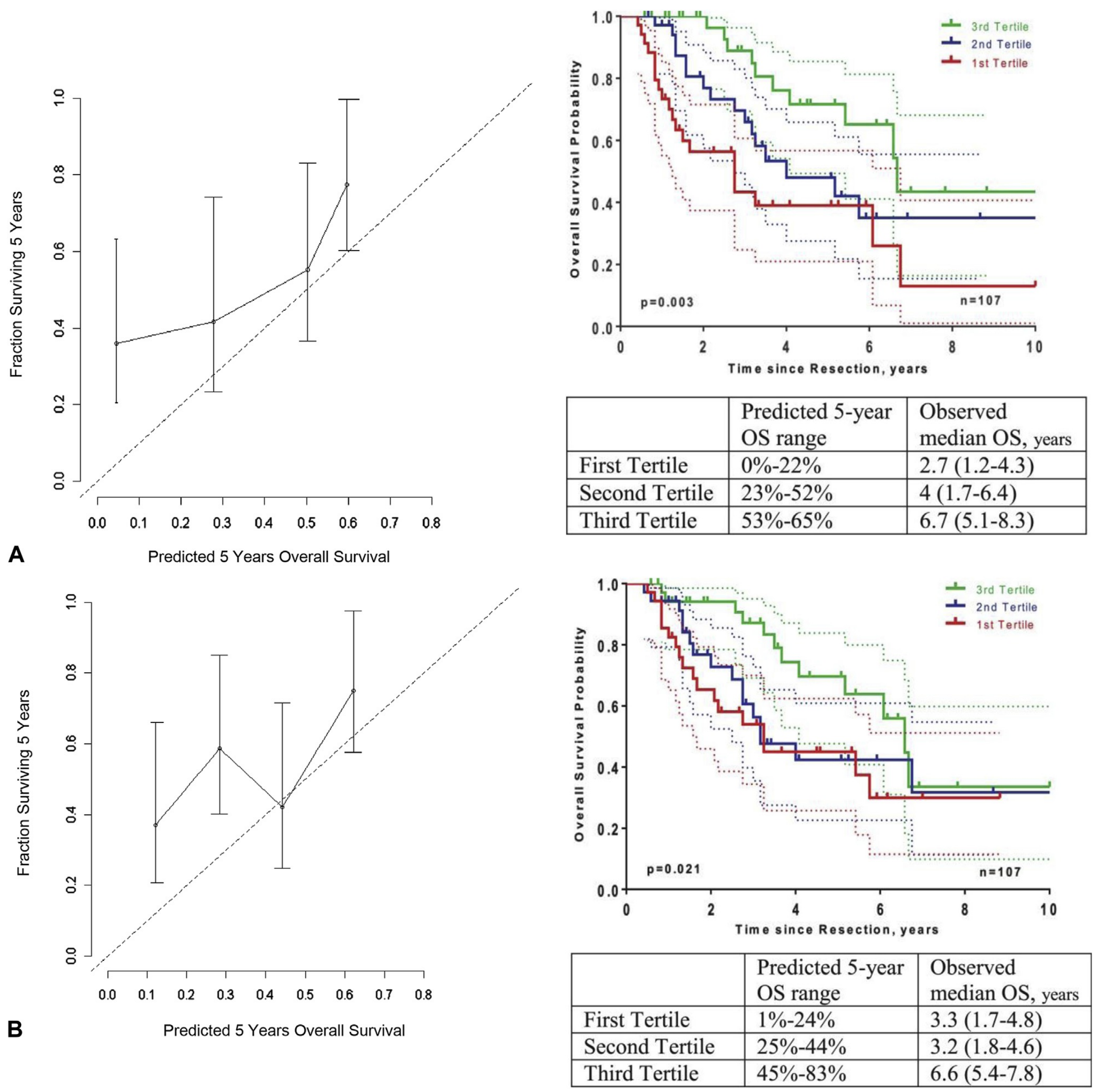
Tableau 1. Détails des items inclus dans la classification AJCC 7ème édition (A), le nomogramme de Wang et al. (B) et celui de Hyder et al. (C)

Analyse statistique

Chaque modèle était appliqué à l'ensemble des patients inclus et ayant toutes les données nécessaires pour application des deux nomogrammes (n=107). Les performances pronostiques étaient mesurées en termes de discrimination, de calibration et de stratification. La discrimination correspond à la probabilité que, dans une paire de patients sélectionnés au hasard, celui qui a la survie la plus courte soit celui qui a la probabilité la plus faible de survie prédite par le nomogramme. Cette probabilité correspond à l'index de concordance (C-index) calculé selon la méthode de Harrell (47). Le seuil de 0,7 est habituellement considéré comme acceptable pour juger de la discrimination d'un modèle pronostique. La calibration consiste, quant à elle, à classer les patients en groupes (tertiles ou quartiles de probabilité de survie prédite par les nomogrammes) selon leurs probabilités de survie globale (courbes de calibration). La stratification consiste ensuite, à comparer la survie observée de ces mêmes groupes (tertiles ou quartiles de probabilité de survie) selon Kaplan-Meier avec la technique du log-rank. Les analyses statistiques ont été réalisé avec les logiciels SPSS version 22.0 et R version 3.1.1.

RESULTATS

Dans la cohorte du MSKCC, le nomogramme de Wang et al, obtenait un C-index de 0,72 (95% CI, 0,64-0,80) et celui de Hyder et al, un C-index de 0,66 (95% CI, 0,56-0,74). La classification AJCC obtenait quant à elle, un C-index de 0,63 (95% CI, 0,58-0,67). Les courbes de calibration et stratification des nomogrammes de Wang et Hyder sont visibles, montrant notamment une stratification optimale (p=0,003 et p=0,021 respectivement, *Figure 1*).



	Predicted 5-year OS range	Observed median OS, years
First Tertile	0%-22%	2.7 (1.2-4.3)
Second Tertile	23%-52%	4 (1.7-6.4)
Third Tertile	53%-65%	6.7 (5.1-8.3)

	Predicted 5-year OS range	Observed median OS, years
First Tertile	1%-24%	3.3 (1.7-4.8)
Second Tertile	25%-44%	3.2 (1.8-4.6)
Third Tertile	45%-83%	6.6 (5.4-7.8)

Figure 1. Courbes de de calibration et stratification des nomogrammes de Wang et al. (A) et de Hyder et al. (B)

Concernant la classification AJCC, la stratification n'était pas optimale puisqu'il n'existait pas de différence significative de survie globale entre les patients de stade II (médiane, 32,7 mois) et stade III (médiane, 51,9 mois) (*Figure 2*).

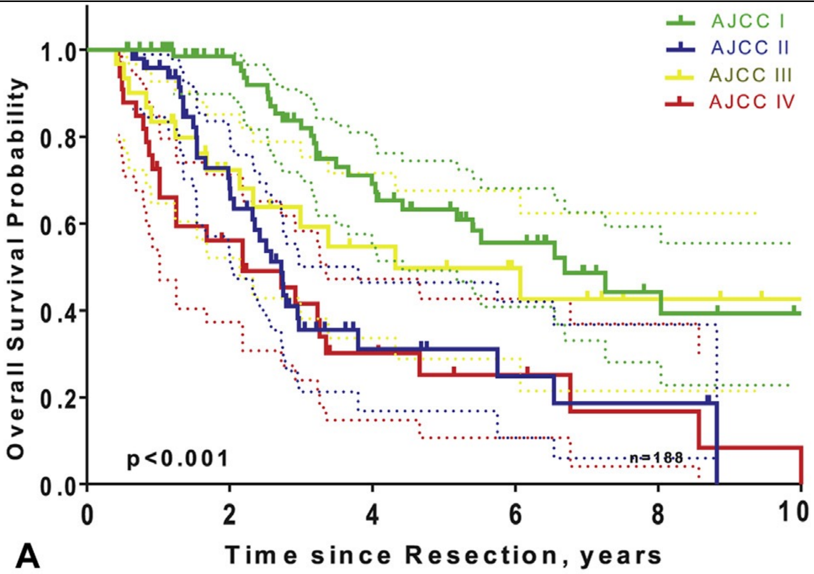


Figure 2. Courbe de stratification de la 7ème édition de la classification AJCC

DISCUSSION

Cette analyse, publiée en 2015 (Annexe 2), était la première à valider de manière externe ces deux nomogrammes. Les nomogrammes représentent une méthodologie pronostique attractive dans le développement d'une médecine personnalisée, actuellement en plein essor. Il est montré ici que le nomogramme de Wang et al, apporte la meilleure estimation pronostique (survie globale) en comparaison aux autres modèles disponibles, dont la classification AJCC 7ème édition. Ces résultats sont en accord avec ceux d'une étude ultérieure rapportant la supériorité pronostique de ce nomogramme vis-à-vis de celui de Hyder et al, et de la plus récente 8ème édition de la classification AJCC (48).

Concernant la 7ème édition de la classification AJCC, elle apporte une estimation pronostique inférieure avec notamment un défaut de stratification entre les stades II et III. Cet élément a également été rapporté dans d'autres études (31,48). Ce défaut est, par ailleurs, toujours présent dans la 8ème édition de l'AJCC, dont l'apport pronostique semble limité (33,34). Toutefois, ce défaut de stratification peut possiblement être expliqué par une évaluation incorrecte du statut ganglionnaire. En effet, plus d'un tiers des patients opérés pour CCIH n'ont pas de curage pédiculaire hépatique associé et sont généralement considérés N0 par défaut, sur la base de l'imagerie préopératoire (37). Hors, l'analyse du statut ganglionnaire par l'imagerie préopératoire est reconnue comme non fiable (49). Ainsi, Farges et al ne retrouvaient pas ce défaut de stratification entre les stades II et III parmi 163 patients ayant tous bénéficié d'un curage pédiculaire hépatique systématique (50). Un curage pédiculaire hépatique systématique augmenterait donc la valeur pronostique de la classification AJCC et est maintenant recommandé comme systématique au cours d'une hépatectomie pour CCIH (51).

Enfin, malgré ses performances diagnostiques, l'applicabilité de ce nomogramme en pratique clinique et notamment sa place dans le processus de prise de décision concernant un éventuel traitement adjuvant reste à définir. Une approche pronostique alternative réside dans l'utilisation d'un arbre décisionnelle thérapeutique basé sur le pronostic. Cette approche a déjà été proposée voire adoptée en pratique courante dans la prise en charge de certains cancers dont le carcinome hépatocellulaire et rapportée pour la prise en charge du CCIH (52–55). Le développement de nouveaux modèles pronostiques, incluant de nouveaux biomarqueurs tumoraux, est maintenant nécessaire.

EVALUATION PRONOSTIQUE PREOPERATOIRE

EVALUATION PRONOSTIQUE PREOPERATOIRE (Annexes 3, 4, 5) [53,56]

METHODES

Population d'étude

Comme précédemment, parmi 199 patients consécutivement opérés d'un CCIH, à visée curative au Memorial Sloan Kettering Cancer Center (MSKCC) (Annexe 1), les patients décédés dans les 90 jours postopératoires ou ayant bénéficié d'une résection palliative (résection R2, carcinose synchrone) étaient exclus de cette analyse.

A partir de cette cohorte,

- 189 patients étaient inclus pour le développement d'un modèle pronostique préopératoire. Ce modèle sera ensuite validé dans la cohorte externe (n=522) de l'AFC (Annexe 3) (53)
- 66 patients étaient inclus pour l'analyse de l'apport pronostique de la génomique tumorale (Annexe 4, données non publiées soumises, Annals of Surgical Oncology, under review)
- 24 patients étaient inclus pour l'analyse de l'apport pronostique des miR circulants (miR21 et miR221) (Annexe 5) (56)

Facteurs pronostiques évalués

Les facteurs cliniques préopératoires inclus dans l'analyse étaient les suivants : âge, sexe, présence d'une maladie hépatobiliaire sous-jacente, taux sérique de CA19-9, taille et nombre tumorale à l'imagerie, suspicion d'invasion ganglionnaire à l'imagerie et traitement néoadjuvant.

Les prélèvements (foie tumoral et non tumoral) compatibles avec une analyse génomique (n=66) étaient retenus pour séquençage génomique de nouvelle génération. En bref, les librairies obtenues par hybridation étaient amplifiées avant d'être soumises à une recherche ciblée de tous les exons et certains introns des 410 gènes sélectionnés, regroupés en voie de signalisation précédemment identifiés dans de nombreux cancers dont le CCIH (57,58). L'association entre altérations génétiques et survie était ensuite évaluée.

Les miR circulants miR21 et miR221 ont été rapporté comme surexprimés dans les tissus tumoraux de CCIH (n=12) et ont permis, dans une cohorte indépendante (n=32), la différentiation diagnostique entre des patients atteints de CCIH et des patients sains. L'association de ces miR avec facteurs tumoraux et survie était ensuite évaluée.

Analyse statistique

Concernant l'analyse de l'apport pronostique des facteurs cliniques préopératoires, une première analyse était réalisée dans la cohorte du MSKCC. Les variables significativement associées à la survie sans récidive en analyse univariée, étaient incluses dans un modèle multivarié de Cox afin d'identifier les variables pronostiques indépendantes. Ensuite, grâce à une méthode de « recursive partitioning », un modèle pronostique sous la forme d'un arbre de classification était développé puis appliqué dans la cohorte multicentrique de l'AFC pour validation externe. En bref, le recursive partitioning est une méthode statistique consistant, à partir d'une population donnée, à développer un arbre de classification utilisant plusieurs variables dichotomiques, pour différencier cette population en groupes les plus différents possibles.

Concernant l'apport pronostique des facteurs génomiques tumoraux et des miR circulants (miR21 et miR 221), leur impact en terme de survie globale et sans récidive était analysé. Survie globale et sans récidive étaient estimées par la méthode de Kaplan-Meier et correspondaient aux intervalles entre résection et dernière date de suivi ou date de récidive, respectivement. Décès ou récidive étaient les événements d'intérêt pour l'analyse de la SSR alors que les patients vivants et sans récidive au dernier suivi étaient censurés. Les différences en termes de survie entre les groupes étaient analysées par test du log rank. Toutes les analyses sus-décrites ont été réalisé avec les logiciels SPSS version 22.0 (SPSS Inc., Chicago, IL) et R, version 3.1.1.

RESULTATS

Facteurs pronostiques préopératoires (Annexe 3) (53)

A partir des 189 patients de la cohorte du MSKCC, la taille et la multifocalité tumorale à l'imagerie étaient identifiées comme facteurs pronostiques indépendamment associés à la survie (*Tableau 2*). Ensuite, par recursive partitioning, un arbre de classification permettait de séparer la cohorte en deux groupes à « faible » et haut risque de récidive (*Figure 3*). La taille tumorale à l'imagerie était le facteur le plus important et l'existence d'une maladie multifocale permettait de séparer plus clairement les patients avec une taille tumorale inférieure. Les deux groupes avaient une médiane de survie sans récidive significativement différente (faible risque vs haut risque, médiane = 31.3 mois vs. 12 mois ; $p<0.001$). Dans la cohorte externe de l'AFC, cette classification permettait à nouveau de stratifier les patients en deux groupes significativement distincts en terme de survie sans récidive (faible risque vs haut risque, médiane = 26 mois vs. 13 mois; $p<0.001$).

Tableau 2. Analyse univariée et multivariée des facteurs préopératoires pronostiques de survie sans récidive à partir des 189 patients de la cohorte MSKCC

	Univariable analysis				Multivariable analysis		
Variable	Median disease-free survival, mo	HR	95% CI	p Value	HR	95% CI	p Value
Preoperative							
Age	—	0.98	0.97–0.99	0.047	0.98	0.97–1.01	0.13
Sex							
Female	23.4	—	—	0.93	—	—	
Male	19.6	—	—		—	—	
Hepatitis							
Yes	16.6	—	—	0.11	—	—	
No	20	—	—		—	—	
PSC/IBD							
Yes	28.1	—	—	0.45	—	—	
No	17.8	—	—		—	—	
Preoperative tumor size	—	1.10	1.05–1.15	<0.001	1.09	1.04–1.14	<0.001*
Preoperative multiple tumor							0.013*
Yes	12	—	—	0.002	1.73	1.12–2.70	
No	23.4	—	—		—	—	
Preoperative enlarged lymph node							
Yes	16.9	—	—	0.66	—	—	
No	19.7	—	—		—	—	
Total bilirubin, mg/L	—	1.03	0.98–1.09	0.224			
Carcinogen antigen, 19-9, U/mL	—	1	1–1	0.004	1	1–1	0.3
Neoadjuvant therapy							
Yes	15.6	—	—	0.32	—	—	
No	20	—	—		—	—	

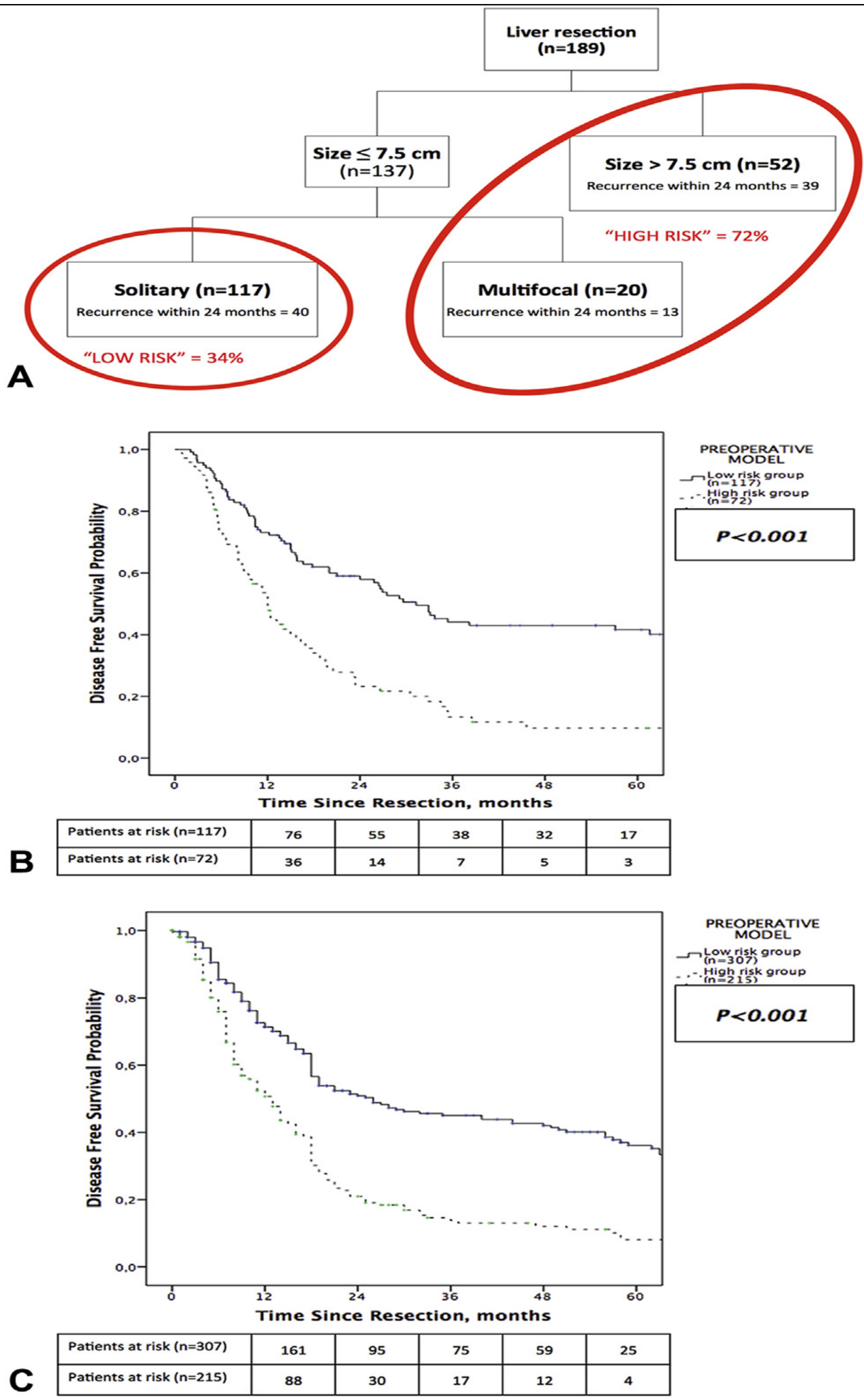
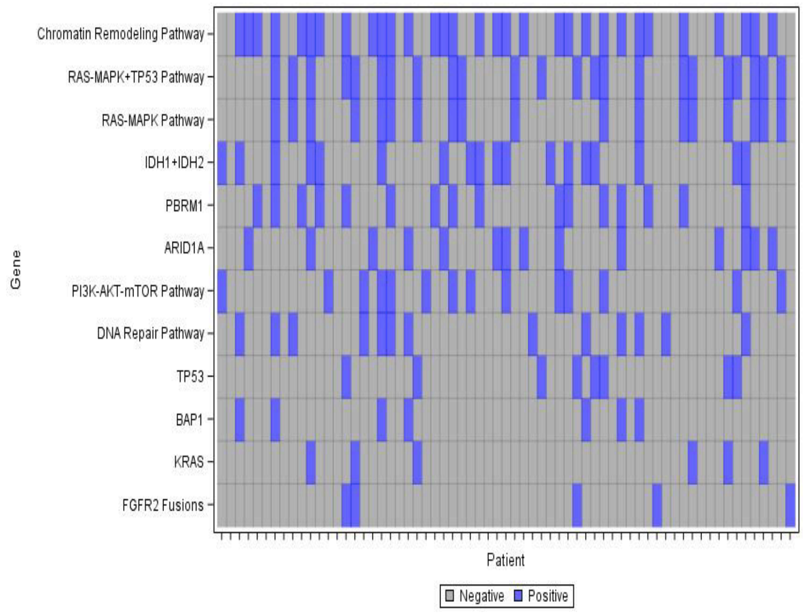


Figure 3. Modèle préopératoire permettant de classifier les patients en deux groupes de risque de récidive (A). Courbes de survie sans récidive selon Kaplan-Meier des patients stratifiés selon le modèle pronostique préopératoire dans la cohorte du MSKCC (B, cohorte de développement) et de l'AFC (C, cohorte de validation).

Apport pronostique de la génomique tumorale (Annexe 4)

Parmi les 66 patients inclus, l'âge médian était de 64,5 ans (min 28,7 - max 86,9), environ un quart des patients avait une maladie multifocale (24,2%) et la taille tumorale maximale médiane était de 6 cm. Le taux de résection R0 était de 78,8%. Parmi les 29 patients ayant bénéficié d'un curage pédiculaire hépatique, 12 présentaient un envahissement ganglionnaire. Enfin, 47 patients (71,2%) ont reçu un traitement périopératoire. Le nombre médian d'altérations génétiques identifiées était de 3 (min 0 – max 26). Les altérations les plus fréquentes concernaient les familles de gènes suivantes (*Figure 4*) :

- remodelage de la chromatine (BAP1, ARID1A, PBRM1) (n=31, 47.0%)
- voie de signalisation des MAP-kinases (RASA1, KRAS, MAPK) (n=27, 40,9%)
- IDH1/2 (n=18, 27,3%)
- voie de signalisation mTOR (PTEN, PIK3CA, MTOR) (n=14, 21,2%).



	N (%)
PBRM1	16 (24.2)
IDH1	15 (22.7)
ARID1A	14 (21.2)
TP53	8 (12.1)
ATM	7 (10.6)
BAP1	7 (10.6)
KRAS	6 (9.1)
RASA1	6 (9.1)
FGFR2	5 (7.6)
FBXW7	3 (4.5)
IDH2	3 (4.5)
NOTCH4	3 (4.5)
PTEN	3 (4.5)
BRAF	2 (3)
MAPK3	2 (3)
NRAS	2 (3)
PIK3CA	2 (3)
PIK3R2	2 (3)
RPTOR	2 (3)
EP300	1 (1.5)
MAP2K1	1 (1.5)
MAP3K13	1 (1.5)
MTOR	1 (1.5)
STK11	1 (1.5)
TSC1	1 (1.5)

Figure 4. Heatmap et liste des altérations génétiques par ordre de fréquence, retrouvées chez 66 patients réséqués.

Concernant l'apport pronostique de l'analyse génomique tumorale, aucune mutation n'était significativement associée à la survie, bien que l'existence d'une mutation des gènes de la famille du remodelage de la chromatine tendait à être associé avec une survie globale et sans récidive estimée plus longue (*Tableau 3* et *Figure 5*).

Tableau 3. Analyse univariée de survie globale et sans récidive en fonction du profil génomique tumoral

	Mutation	#T(#E)	SSR 5ans	[95%	CI]	p-value	#T(#E)	SG5ans	[95%	CI]	p-value
IDH1+IDH2	Présent	18 (12)	0.37	[0.14	0.61]	0.23	18 (7)	0.71	[0.44 -	0.87]	0.31
	Absent	48 (39)	0.21	[0.10	0.35]		48 (26)	0.41	[0.25 -	0.57]	
Chromatin Remodeling Gene Family	Présent	31 (20)	0.32	[0.15	0.51]	0.13	31 (12)	0.60	[0.35 -	0.77]	0.09
	Absent	35 (31)	0.20	[0.09	0.36]		35 (21)	0.38	[0.20 -	0.56]	
RAS-MAPK +TP53 Pathway	Présent	24 (21)	0.21	[0.07	0.40]	0.23	24 (16)	0.29	[0.11 -	0.50]	0.29
	Absent	42 (30)	0.29	[0.15	0.45]		42 (17)	0.61	[0.42 -	0.76]	
PI3K-AKT-mTOR Pathway	Présent	14 (13)	0.21	[0.05	0.45]	0.23	14 (9)	0.27	[0.07 -	0.53]	0.31
	Absent	52 (38)	0.28	[0.15	0.41]		52 (24)	0.55	[0.38 -	0.70]	
DNA Repair Gene Family	Présent	14 (11)	0.29	[0.07	0.55]	0.65	14 (6)	0.66	[0.32 -	0.86]	0.33
	Absent	52 (40)	0.26	[0.14	0.39]		52 (27)	0.43	[0.27 -	0.59]	

#T nombre total de patients; #E, nombre de patients ayant présenté l'évènement correspondant (décès ou récidive); CI, intervalle de confiance

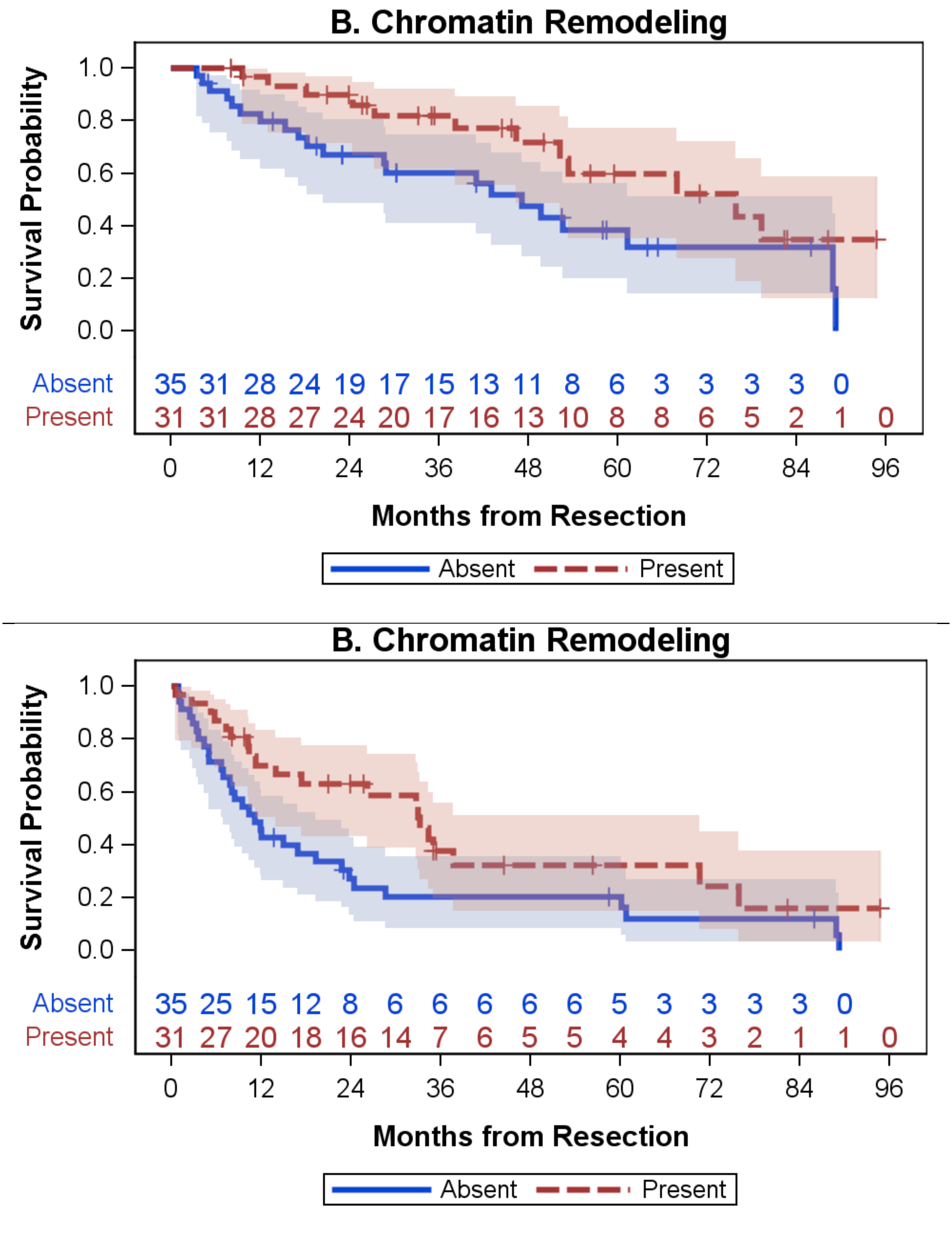


Figure 5. Estimations selon Kaplan-Meier de survie globale (en haut) et sans récidive (en bas) selon l'existence ou non de mutations des gènes impliqués dans le remodelage de la chromatine

Apport pronostique des miR tumoraux circulants (Annexe 5) (56)

Parmi les 24 patients inclus, l'âge médian était de 64 ans (min 28,7 - max 86,9). Deux patients avaient une maladie multifocale (9%) et la taille tumorale maximale médiane était de 5,6 cm. Le taux de résection R0 était de 95,8%. Parmi les 10 patients ayant bénéficié d'un curage pédiculaire hépatique, 2 (20%) présentaient un envahissement ganglionnaire. Enfin, six patients (25%) ont reçu un traitement périopératoire.

Concernant les caractéristiques tumorales, le miR221 circulant était significativement surexprimé chez les patients avec une tumeur peu à non différencié (n=5) en comparaison aux patients avec tumeur moyennement à bien différencié (n=19, p=0.016), sans être toutefois associé à la survie globale et sans récidive. Concernant miR21, son expression tendait à être moins importante dans le groupe de patients survivant moins de 24 mois sans atteindre une différence statistiquement significative (p=0.087, *Figure 6*).

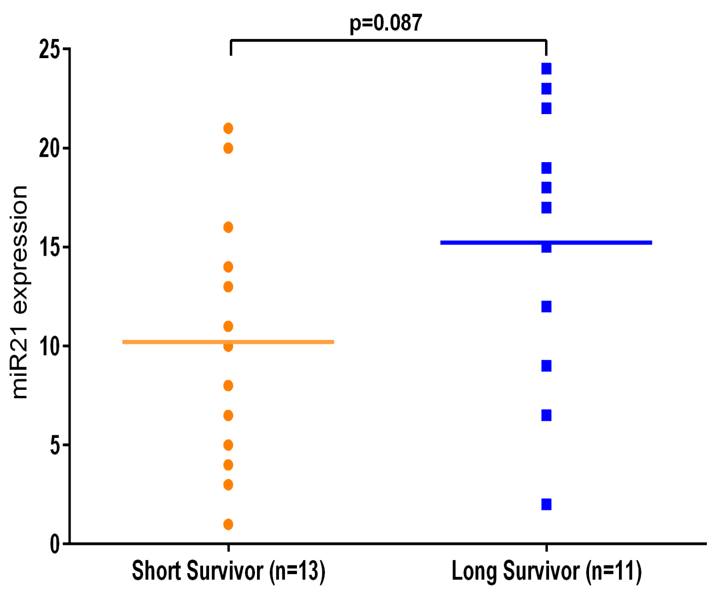


Figure 6. Expression du miR21 au niveau circulant chez les patients survivants moins de 2 ans (short) et plus de 2 ans (long)

DISCUSSION

La résection chirurgicale complète étant le seul traitement curatif du CCIH, l'estimation préopératoire du pronostic est rarement considérée en cas de maladie résécable. L'utilisation du modèle préopératoire développé ici, à partir de données de l'imagerie, permet toutefois d'identifier deux groupes distincts en terme de risque de récidive précoce et donc de survie (sans récidive et globale).

Dans notre étude, l'existence d'une maladie multifocale était associée avec un risque de récidive à 24 mois de 117% par rapport à une maladie unifocale (53). Ce résultat est en accord avec l'étude de Nam et al. proposant un nomogramme préopératoire prédictif de la futilité de la chirurgie pour CCIH incluant la multifocalité tumorale comme facteur pronostique majeur (HR=5,987, 95%CI 2,643-13,887 ; p<0,001) (59). De la même manière, Spolverato et al. ont rapporté qu'en cas de maladie multifocale, la probabilité de guérison est de seulement 12,6% (60). L'existence d'une maladie multifocale à l'imagerie pourrait ainsi constituer un élément pronostique majeur à considérer dans la prise en charge thérapeutique. Enfin, l'analyse qualitative et quantitative de l'imagerie préopératoire telle l'analyse de texture pourrait permettre de générer de potentiels nouveaux marqueurs pronostiques (61–63). Concernant le CA19-9 préopératoire, il n'apparaissait pas comme facteur indépendant de survie dans notre étude et son apport pronostique en cas de résécabilité reste débattu bien qu'il soit classiquement élevé en cas de maladie avancée (60,64).

Avec l'avènement du séquençage génomique de nouvelle génération, l'analyse génomique des CCIH a permis l'identification de potentielles cibles thérapeutiques ayant conduit à plusieurs essais thérapeutiques actuellement en cours (65). Toutefois, à l'inverse d'autres tumeurs solides, le CCIH n'est pas associé à une altération génétique spécifiquement récurrente ou une voie de signalisation promotrice préférentielle. A partir d'une cohorte de patients réséqués, les altérations génétiques les plus fréquemment retrouvés concernaient la famille des gènes impliqués dans le remodelage de la chromatine (47,0%), la voie de signalisation des MAP-kinases (40,9%) et IDH1/2 (27,3%), correspondant ainsi aux résultats les plus fréquemment rapportés (66–68). En revanche, aucune altération génétique dont celle concernant FGFR2, IDH1/2 ou KRAS n'était significativement associée à la survie. Ces résultats contrastent avec ceux de précédentes études (43,67,69). Ces données contradictoires peuvent être expliquées par une hétérogénéité notable entre les cohortes d'étude, incluant des proportions inégales de patients avec maladie résécable et non résécable et recevant des traitements systémiques variables. La cohorte utilisée ne concerne ici que des patients ayant eu une résection. L'absence d'association significative entre altérations génétiques et survie peut également s'expliquer par le fait qu'en cas de résection complète, le pronostic est conditionné par les caractéristiques histopathologiques tumorales au moment de la résection qui priment alors sur la génomique tumorale. En d'autres termes, la résection impacte le pronostic en modifiant l'histoire naturelle de la tumeur classiquement conditionnée par la biologie tumorale en l'absence de résection. Toutefois, Sia et al. ont identifié à partir de 153 patients réséqués, deux profils génomiques distincts, respectivement « inflammatoire » et « prolifératif », associés à des survies et des profils de récidive après résection significativement distincts (70). Sous réserve de confirmation dans une cohorte externe prospective, ces données pourraient être intégrées à l'estimation du pronostic après résection.

Les miR tumoraux circulants représentent un autre outil pronostique préopératoire de choix puisqu'accessibles par simple prise de sang. Le travail exploratoire rapporté ici est intéressant puisqu'outre son intérêt diagnostique, la surexpression du miR221 était significativement associée au degré de différentiation tumorale sans être toutefois associée avec la survie. Concernant miR21, il tendait à être exprimé différemment entre les patients survivants moins ou plus de 24 mois sans pour autant corréler avec l'histologie tumorale. Ces deux miRs ont déjà été décrits comme promoteur de tumeur en inhibant des gènes suppresseurs de tumeur comme PTEN, PDCD4 ou mTOR et p53, mais ils n'ont pas été identifiés comme facteur pronostique dans la cohorte d'étude (71–74). Alors que miR221 n'a été que peu étudié dans le CCIH, les résultats concernant miR21 s'opposent à d'autres études rapportant l'implication de miR21 dans les phénomènes de prolifération et d'invasion de cellules de cholangiocarcinome in vitro et in vivo (75,76). Les résultats rapportés ici doivent être considérés avec précaution. Tout d'abord, la taille de la cohorte d'étude représente une première limite. De plus, la prise en charge périopératoire des patients au sein de la cohorte était assez hétérogène. Enfin, seul l'intérêt pronostique de miR21 et miR221 était exploré alors que d'autres miR ont été précédemment rapporté comme potentiel marqueur pronostique (77,78). Par ailleurs, d'autres marqueurs circulants également accessibles par simple « biopsie liquide » pourraient être considérés comme outil pronostique préopératoire et n'ont pas été évalués dans cette cohorte (79–81).

EVALUATION DE L'IMPACT PRONOSTIQUE DE LA TRANSFUSION PEROPERATOIRE ET DE LA MORBIDITE PERIOPERATOIRE

EVALUATION DE L'IMPACT PRONOSTIQUE DE LA TRANSFUSION PEROPERATOIRE ET DE LA MORBIDITE PERIOPERATOIRE (Annexes 6,7) [82,83]

METHODES

Population d'étude

Parmi les 581 patients de la cohorte AFC opérés d'un CCIH à visée curative, tous les patients décédés dans les 90 jours postopératoires ou ayant bénéficié d'une résection palliative (résection R2, carcinose synchrone) étaient exclus. La cohorte AFC était ainsi constituée de 522 patients (Annexe 1).

Définitions

La transfusion peropératoire était définie par l'administration intraveineuse, durant la résection, de concentrés globulaires. L'utilisation de plasma frais congelé et concentré plaquettaire n'était pas considérée dans l'analyse. Tout au long de la période d'étude, l'usage de transfusion reposait sur les recommandations de la Société Française d'Anesthésie Réanimation (84).

Concernant la morbidité, tout événement déviant de suites opératoires normales était considéré comme une complication et gradé selon la classification de Dindo-Clavien (85). Une complication de grade supérieur à II était considérée comme sévère. En cas de complications multiples, le grade de la complication la plus élevée était retenu. Les complications spécifiques à la chirurgie hépatique, telles que insuffisance hépatocellulaire, fistule biliaire et hémorragie, répondaient aux définitions de l'International Study Group for Liver Surgery (86–88).

Analyse statistique

L'association entre transfusion (Annexe 6) ou morbidité (Annexe 7) et survie étaient d'abord analysée en univariée puis dans un modèle multivarié de Cox afin d'ajuster pour de potentiels facteurs confondants. Concernant l'analyse de l'impact pronostique de la transfusion peropératoire, elle était réalisée après appariement 1:1 par score de propension.

Les survies globale et sans récidive étaient estimées par la méthode de Kaplan-Meier et correspondaient aux intervalles entre résection et dernière date de suivi ou date de récidive, respectivement. Décès et décès ou récidive étaient les événements d'intérêt pour l'analyse de la SG et SSR respectivement. Toutes les analyses ont été réalisées avec les logiciels SPSS version 22.0 er 23.0 (SPSS Inc., Chicago, IL).

RESULTATS

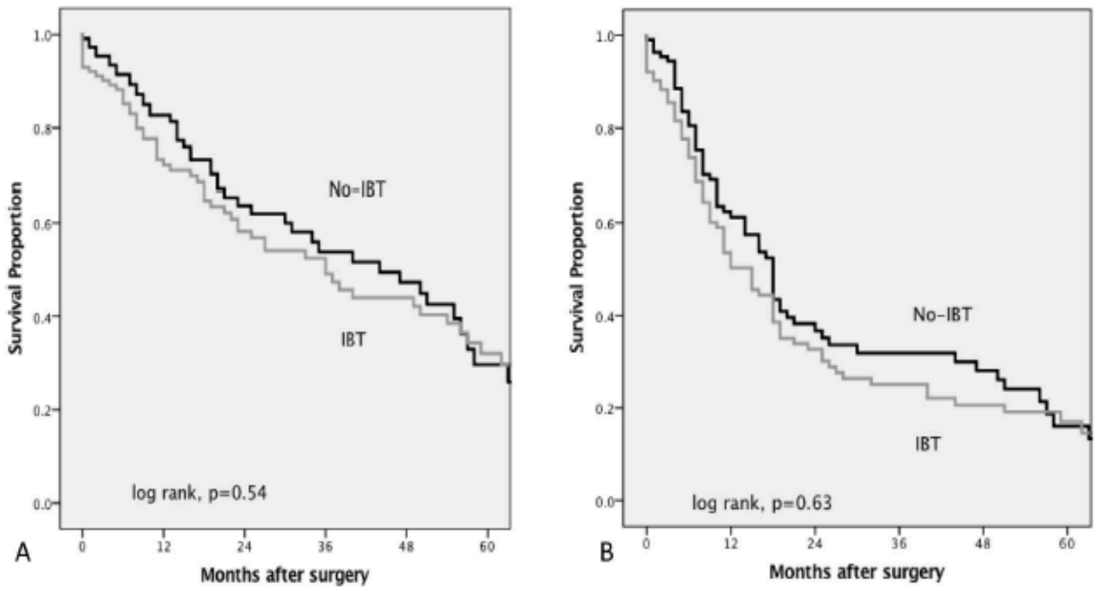
Impact pronostique de la transfusion peropératoire (Annexe 6) (82)

Parmi les 522 patients de la cohorte AFC, 98 patients ayant reçu une transfusion peropératoire étaient appariés à 98 patients non transfusés par score de propension avec un calibrage à 0,01 permettant d'obtenir deux groupes comparables (p>0.02) (*Tableau 3*). Aucune différence de SG ou SSR n'était retrouvée entre ces deux groupes (*Figure 7*).

Tableau 3. Caractéristiques des deux groupes de patients ayant reçu (n=98) ou non (n=98) une transfusion peropératoire après appariement avec score de propension

	No-IBT n = 98	IBT n = 98	p
Patient characteristics			
Male Gender	52 (53%)	48 (49%)	0.667
Age, years	64 (11.8)	64 (11.2)	0.970
BMI, kg/m^2	24.7 (3.7)	24.7 (4.2)	0.853
ASA Score >2	12 (12%)	7 (7%)	0.330
Neoadjuvant chemotherapy	6 (6%)	8 (8%)	0.780
Preoperative PVE	5 (5%)	6 (6%)	>0.999
Operative Data			
Resection period			>0.999
1989–1999	33 (40%)	34 (35%)	
2000–2009	65 (66%)	64 (65%)	
Hospital IHCC resection/year volume/year	3.1 (2.14)	3.4 (2.61)	0.840
Major Hepatectomy	81 (83%)	77 (78%)	0.581
Portal lymphadenectomy	51 (52%)	46 (47%)	0.566
Combined vascular resection	8 (8%)	8 (8%)	>0.999
Common bile duct resection	22 (22%)	17 (17%)	0.470
Associated extrahepatic resection	6 (6%)	7 (7%)	>0.999
Vascular clamping	80 (82%)	82 (84%)	0.850
Tumor characteristics			
Tumor size, cm	7.0 (3.5)	6.8 (3.4)	0.750
Multifocal disease on specimen	34 (35%)	37 (38%)	0.763
Vascular invasion	42 (43%)	39 (40%)	0.770
Perineural Invasion	24 (24%)	26 (26%)	0.870
Nodal status			0.570
N0	37 (38%)	37 (38%)	
N1	14 (14%)	39 (40%)	
Nx	47 (48%)	26 (26%)	
Resection margin status			0.750
R1	70 (71%)	67 (68%)	
Adjuvant Chemotherapy	39 (40%)	41 (42%)	0.883

Data are presented as mean (standard deviation) or n (%) as appropriate. Abbreviations: No-IBT, no intraoperative transfusion; IBT, intraoperative transfusion; ASA, American Society of Anesthesiologists; BMI, Body Mass Index; PSM, propensity score matching; PVE, portal vein embolization.



Patients at risk	Total	1 year	2 year	3 year	4 year	5 years
No IBT	98	85	53	35	21	15
IBT	98	89	60	38	30	20

Patients at risk	Total	1 year	2 year	3 year	4 year	5 years
No IBT	98	85	53	35	21	15
IBT	98	89	60	38	30	20

Figure 7. Estimation de survie globale (A) et sans récidive (B) selon Kaplan-Meier des deux groupes de patients appariés avec score de propension, ayant reçu (n=98) ou non (n=98) une transfusion peropératoire.

Impact pronostique de la morbidité postopératoire (Annexe 7) (83)

Parmi les 522 patients de la cohorte AFC, 222 présentèrent une complication (42,5%) avec un taux de complication sévère de 21,6% (n=113). Cinquante-cinq patients (10,5%) présentèrent plusieurs complications. La survenue de complications sévères était associée à une SG plus courte en comparaison à l'absence de complications ou la survenue de complications mineures ainsi qu'à une SSR plus courte en comparaison à l'absence de complications (*Figure 8*).

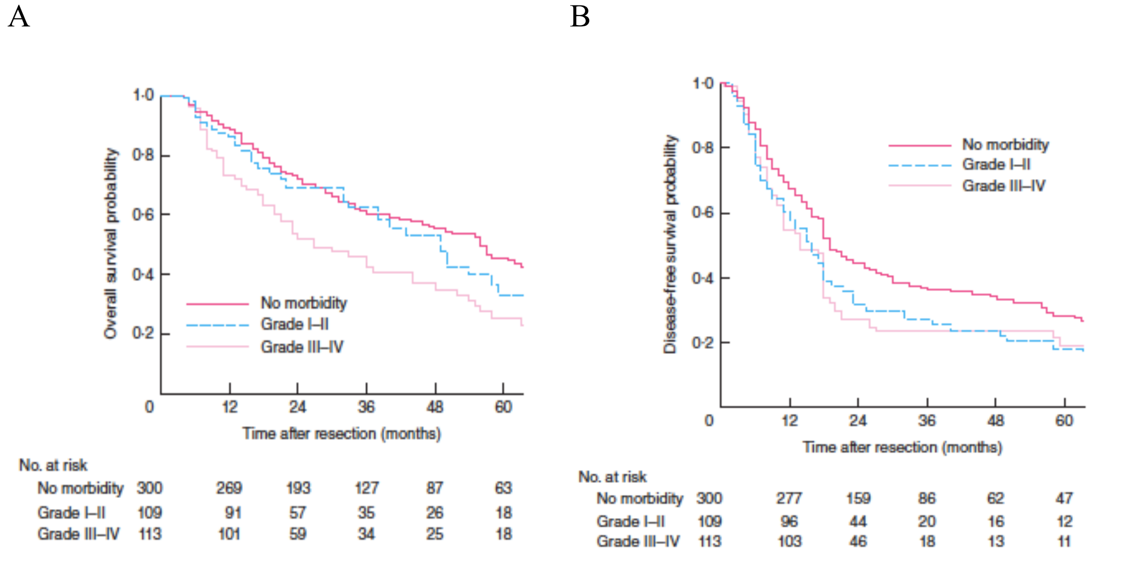


Figure 8. Estimation de survie globale (A) et sans récidive (B) selon Kaplan-Meier des patients en fonction de la survenue de morbidité postopératoire

En analyse multivariée, la survenue de complications sévères était indépendamment associée à la SG (HR=1,64 ; 95%CI 1,21-2,23 ; p=0,002) mais pas à la SSR (HR=1,15; 95%CI 0,88 -1,50; p=0,310). Toutefois, parmi les 248 patients qui avaient présenté une récidive, la survenue d'une complication sévère était indépendamment associée à un délai de récidive plus court.

DISCUSSION

Le recours à la transfusion a longtemps été considéré comme associé à une survie réduite après résection de nombreuses tumeurs malignes (89,90). Cette association a souvent été démontrée par comparaison de groupes significativement différents, notamment en termes de caractéristiques tumorales. Dans la cohorte d'étude, les patients transfusés avaient une maladie plus souvent multifocale avec une taille tumorale plus importante augmentant la nécessité de clampage et résection vasculaire. Afin de s'affranchir de ces facteurs confondants, il a été réalisé un appariement par score de propension montrant l'absence d'impact pronostique de la transfusion. En revanche, toujours en utilisant deux groupes appariés par score de propension, le recours à la transfusion était significativement associé à un risque accru de morbidité sévère. En utilisant la même approche, Yang et al ont également montré l'absence d'impact pronostique de la transfusion après hépatectomie pour carcinome hépatocellulaire (91). Bien qu'associée à l'existence d'une maladie avancée nécessitant une résection complexe, la transfusion peropératoire n'est donc pas directement associée au pronostic du CCIH réséqué mais plutôt à la survenue de morbidité sévère. Une politique restrictive de transfusion doit donc être préférée. Alors qu'une limite de ce travail est l'absence d'analyse de l'impact de la transfusion périopératoire, le timing peropératoire est généralement reconnu comme le plus pertinent concernant l'impact de la transfusion sur les suites à long terme en chirurgie cancérologique (92).

A partir de cette large cohorte rétrospective multicentrique, la morbidité postopératoire était observée dans 40% des cas et la moitié était sévère alors que 8% des patients étaient décédés à 90 jours postopératoires. Ces observations confirment que la résection complète des CCIH est le plus souvent complexe, s'associant ainsi avec un risque important de morbimortalité. De plus, comme précédemment rapportés pour d'autres tumeurs malignes hépatiques, la morbidité postopératoire impactait significativement la survie à long terme (93–95). De manière intéressante, la morbidité sévère était indépendamment associée à un délai de récidive plus court mais n'était pas indépendamment associée à une SSR plus courte. Cette observation suggère que la survenue d'une récidive est principalement conditionnée par la biologie tumorale mais que la survenue d'une complication pourrait accélérer la survenue de la récidive au cours des 12 premiers mois, comme rapporté pour d'autres tumeurs comme l'adénocarcinome œsogastrique (96).

DISCUSSION GENERALE ET PERSPECTIVES

DISCUSSION GENERALE

Les facteurs cliniques pronostiques traditionnels du CCIH réséqué sont mieux connus. Mais progressons-nous vers une meilleure compréhension de cette maladie ? (97). Même en restant limité aux caractéristiques histopathologiques tumorales traditionnellement utilisées, les modèles pronostiques actuels évoluent. Les divers modèles publiés incluent différents paramètres, leur attribuant une valeur pronostique variable. Pour exemple, alors qu'elle est reconnue comme facteur pronostique majeur dans le cadre de nombreuses tumeurs malignes dont le CCIH, la taille tumorale vient seulement d'être incluse dans la 8ème édition de la classification AJCC en utilisant une limite de 5 cm pour définir ainsi les stades Ia et Ib (98). Malgré tout, ce seuil de 5 cm reste discutable. Deux études utilisant des approches statistiques différentes au sein de larges cohortes ont conclu que la taille tumorale permettant une stratification pronostique plus précise serait plutôt située autour de 7 cm (29,53). De plus, une récente étude s'intéressant aux patients survivants plus de 5 ans montrait qu'une proportion de ces patients (<10%) présentait des facteurs histologiques pronostiques péjoratifs tels qu'une maladie multifocale et un envahissement ganglionnaire pN1, classiquement reconnus comme incompatibles avec une survie à long terme (10). Ces observations confirment que les facteurs pronostiques traditionnels ont un impact variable entre les différentes cohortes d'étude et surtout, ils ne donnent qu'une indication parcellaire de l'évolution de la maladie. Une meilleure connaissance de la biologie tumorale est indispensable pour estimer avec fiabilité le pronostic de cette maladie et ainsi améliorer la sélection des patients avant chirurgie et leur prise en charge périopératoire.

PERSPECTIVES

L'analyse du profil génomique tumoral ainsi que les marqueurs tumoraux circulants et la radiogénomique préopératoire représentent un réel champ d'investigation pour améliorer l'appréciation de la biologie tumorale (61). Toutefois, du fait de la relative rareté du CCIH, seule une initiative prospective multicentrique, idéalement internationale, permettrait d'obtenir suffisamment de données cliniques et biologiques exploitables. Par ailleurs, les deux cohortes utilisées pour l'ensemble de ces travaux sont constituées seulement de patients occidentaux. L'extrapolation de nos résultats à une population orientale où l'incidence, les étiologies et la physiopathologie du CCIH sont différentes reste délicate et souligne l'intérêt d'initiatives internationales futures (99).

Enfin, outre l'amélioration de notre connaissance des facteurs et modèles pronostiques, le développement de nouvelles séquences thérapeutiques reste essentiel afin d'améliorer le pronostic à long terme du CCIH après résection, qui est actuellement semblable à celui de l'adénocarcinome du pancréas (100). Le premier élément de réflexion correspond au profil de récidive qui reste majoritairement intrahépatique et précoce puisque survenant classiquement dans un délai inférieur à 2 ans (9,53). Au vu de ces données, un traitement locorégional hépatique, adjuvant ou néoadjuvant, pourrait constituer une option de choix à ajouter dans la séquence thérapeutique. En traitement adjuvant, une expérience rétrospective monocentrique décrivant l'usage de la chimioembolisation en adjuvant chez 122 patients suggérait un bénéfice chez les patients à haut risque de récidive (101). De plus, les données combinées de deux essais de phase II évaluant la combinaison de floxuridine par voie intra-artérielle hépatique avec une chimiothérapie systémique pour maladie localement irrésécable ou métastatique ont montré un taux de réponse atteignant 59%

avec plusieurs cas de résection secondaire avec réponse histologique complète (7,102). Bien qu'il soit difficile d'extrapoler ces résultats en condition adjuvante, cette approche pour augmenter le contrôle de la maladie notamment au niveau hépatique reste attractive et un essai de phase I est actuellement en cours au MSKCC. Le second élément repose sur l'analyse du profil génomique tumoral. Alors que le nombre moyen d'altérations génétiques retrouvées était de 3 dans la cohorte étudiée et qu'aucune voie de signalisation préférentielle n'a été décrite, différentes cibles thérapeutiques potentielles précédemment décrites dans d'autres cohortes, telles que la mutation des gènes FGFR2 et IDH1-2, ont été retrouvé dans la cohorte étudiée. Les premières données des essais de phase I/II les plus récents montrent des résultats prometteurs en terme de tolérance et réponse et soulignent l'importance des analyses tumorales notamment génomiques dans l'amélioration du pronostic (103). Sans l'élargissement de l'arsenal et des séquences thérapeutiques actuellement à disposition, aucune amélioration pronostique ne pourra être obtenue. La capacité à évaluer le pronostic, que ce soit en préopératoire ou postopératoire, constitue dans ce contexte une pierre angulaire pour décider de l'inclusion des patients dans les essais thérapeutiques à venir autour du CCIH.

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ANNEXES

Annexe 1. Données descriptives des cohortes utilisées.

Variable	Memorial Sloan Kettering Cancer Center cohort (n = 189)	Association Française de Chirurgie cohort (n = 522)	p Value
Preoperative			
Age at surgery, y, mean (SD)	65.4 (11.8)	64 (11.7)	0.35
Female, n (%)	114 (60.3)	268 (51.3)	0.04
Hepatitis, n (%)	18 (9.5)	32 (6.1)	0.14
Hepatitis B virus	9 (4.8)	NA	
Hepatitis C virus	9 (4.8)	NA	
PSC/IBD, n (%)	7 (3.7)	NA	
Imaging modality, n (%)			
CT	170 (89.9)	NA	
MRI	114 (60.3)	NA	
Ultrasound	70 (37)	NA	
PET	59 (31.2)	NA	
Preoperative tumor size, cm, mean (SD)	6.5 (3.6)	6.8 (3.8)	0.16
Preoperative multiple tumor, n (%)	33 (17.5)	79 (15.1)	0.49
Preoperative enlarged lymph node, n (%)	16 (8.5)	NA	
Total bilirubin, mg/L, mean (SD)	1.2 (3.1)	1.55 (3.4)	<0.001
Carcinogen antigen 19-9, U/mL, mean (SD)	1,847.7 (5,354.1)	1,547 (7,101)	0.001
Neoadjuvant therapy, n (%)	10 (5.3)	34 (6.5)	0.6
Postoperative			
Major resection, n (%)	124 (65.6)	401 (76.8)	0.004
Tumor size, cm, mean (SD)	6.9 (3.9)	7.1 (4)	0.9
Multiple lesions, n (%)	54 (28.6)	187 (35.8)	0.08
Underlying liver, n (%)			0.053
Steatosis	69 (36.5)	142 (27.2)	
Cirrhosis	9 (4.8)	25 (4.8)	
Vascular invasion, n (%)			0.6
Absent	121 (64)	321 (61.5)	
Present	68 (36)	201 (38.5)	
Microvascular	46 (24.3)	NA	
Macrovascular	22 (11.6)	NA	
Perineural invasion, n (%)	54 (28.6)	124 (23.8)	0.21
Extrahepatic invasion, n (%)*	22 (11.6)	34 (6.5)	0.012
Morphologic subtype, n (%)			<0.001
Mass-forming	176 (93.1)	367 (70.3)	
Periductal invasion	13 (6.9)	9 (1.7)	
Intraductal growth	—	6 (1.1)	
Mixed subtype	—	58 (11.1)	
Unknown	—	82 (15.7)	
Margin status, n (%)			0.006
Negative	152 (80.4)	365 (69.9)	
Positive	37 (19.6)	157 (30.1)	
pN stage, n (%)			0.22
pNx	97 (51.3)	246 (47.1)	
pN0	71 (37.6)	191 (36.6)	
pN1	21 (11.1)	85 (16.3)	
Adjuvant therapy, n (%)	51 (27)	178 (34.1)	0.084

*Gallbladder excluded.
IBD, inflammatory bowel disease; PSC, primary sclerosing cholangitis.

Annexe 2.

Outcomes after Resection of Intrahepatic Cholangiocarcinoma: External Validation and Comparison of Prognostic Models

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BACKGROUND: Published prognostic models for overall survival after liver resection for intrahepatic cholangiocarcinoma require external validation before use in clinical practice.

STUDY DESIGN: From January 1993 to May 2013, consecutive patients who underwent resection of intrahepatic cholangiocarcinoma were identified from a prospective database. The Wang nomogram was derived in an Asian cohort (n = 367) and included clinicopathologic variables and preoperative CEA and cancer antigen 19-9 levels. The Hyder nomogram was derived in an Eastern and Western multicenter cohort (n = 514) using clinicopathologic variables only. The AJCC Cancer Staging System (7th ed) and the preoperative Fudan risk score were also evaluated. Prognostic performance was assessed in terms of discrimination, calibration, and stratification.

RESULTS: One hundred and eighty-eight patients were included, with a median follow-up of 41 months. Median overall survival was 48.7 months and estimated 3-year and 5-year overall survival rates were 59% and 45%, respectively. Overall survival prediction accuracy, according to concordance-index calculation, was 0.72 with the Wang nomogram, 0.66 with the Hyder nomogram, 0.63 with the AJCC system, and 0.55 using the Fudan score. Both nomograms provided effective patient stratification in distinct survival groups.

CONCLUSIONS: Both the Wang and Hyder nomograms provided accurate patient prognosis estimation after liver resection for intrahepatic cholangiocarcinoma and can be useful for decision making about adjuvant therapy. The Wang nomogram appears to be more appropriate in patients undergoing formal portal lymphadenectomy and requires preoperative CEA and cancer antigen 19-9 levels for optimal performance. (J Am Coll Surg 2015;221:452−461. © 2015 by the American College of Surgeons)

Intrahepatic cholangiocarcinoma (ICC) is the second most common primary hepatic malignancy, with an incidence in the United States of about 1 per 100,000.[1] Although ICC is much less common than hepatocellular carcinoma, its age-adjusted incidence has risen by 165% during the last 30 years, from 0.32 per 100,000 to 0.85 per 100,000.[2,3] The only potentially curative treatment is complete resection, which offers a median overall survival (OS) of about 30 months.[4-7] Adjuvant or neoadjuvant therapy might improve survival after resection, although this hypothesis is based mainly on extrapolation of data from 2 randomized controlled trials for biliary cancers in the palliative setting.[8-10] Prognostic models could potentially optimize identification of patients most likely to benefit from such treatment.

The 7th edition of the AJCC Cancer Staging System introduced a separate TNM classification for ICC; earlier versions did not differentiate between hepatocellular cancer and ICC.[2] Factors included in the AJCC staging for ICC are the number of tumors, vascular invasion, direct invasion of extrahepatic structures, periductal invasion

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Abbreviations and Acronyms

CA 19-9	= cancer antigen 19-9
C-index	= concordance index
ICC	= intrahepatic cholangiocarcinoma
LN	= lymph node
MSKCC	= Memorial Sloan Kettering Cancer Center
OS	= overall survival

(vs mass-forming lesions), lymph node (LN) metastasis, and distant metastasis. Several studies found additional prognostic factors, including positive surgical margin, tumor size, tumor differentiation, and patient age.[11-13] Prognostic nomograms, including such additional variables, might therefore be more accurate than the conventional AJCC staging system for predicting outcomes.[14]

Recently, 1 preoperative prognostic score and 2 prognostic nomograms were published,[7,15,16] but none of these models have been externally validated. The aim of this study was to evaluate and validate the existing prognostic scores for OS after resection of ICC in a large, single-center cohort.

METHODS

Study population

The IRB at Memorial Sloan Kettering Cancer Center (MSKCC) approved this study. All patients that were included underwent a liver resection, and ICC was confirmed at pathologic evaluation of the resected specimen. Lymphadenectomy was performed at the discretion of the surgeon, as either formal peripancreatic and portocaval LN dissection or targeted excision, based on preoperative imaging and intraoperative findings. Resections were extended to extrahepatic structures when required to achieve a macroscopically complete resection.

Perioperative data

Clinical preoperative variables included demographics, preoperative tumor markers (CEA, cancer antigen 19-9 [CA 19-9], and α-fetoprotein), and the time interval between diagnosis and resection. The number of liver lesions and the diameter of the largest tumor were evaluated using preoperative CT, MRI, and intraoperative ultrasonography. Tumor boundary type, as defined and adopted in the Fudan score, was assessed on preoperative cross-sectional imaging.[15]

Pathologic assessment

Pathologic variables included size and number of tumors, differentiation grade, resection margin status, vascular invasion, perineural invasion, number, sites and involvement of harvested LN, and histology of nontumoral liver parenchyma.[2] Extrahepatic involvement was defined as direct invasion of any extrahepatic organs, excluding the gallbladder (pT3). Morphologic subtype was defined as mass-forming, periductal infiltrating, and mixed.[17,18]

Follow-up

Clinical and radiographic monitoring was performed every 4 to 6 months. Adjuvant therapy was offered, at the discretion of the multidisciplinary team, to patients at high risk for recurrence, especially in case of LN-positive disease, vascular invasion, or R1 resection.

Nomograms and risk scores validation

The Wang nomogram was published in 2013 on a derivation cohort of 367 ICC patients resected at a single Asian center.[7] Preoperative CEA and CA 19-9 levels, as well as tumor size, were included as linear continuous variables. Additional dichotomous variables were vascular invasion (no/yes), nodal status (pN0/pN1), and direct invasion or local metastasis (no/yes). The nomogram concordance index (C-index) was 0.75 (95% CI, 0.68−0.83) in a subsequent validation cohort (n = 82) from the same center.

The Hyder nomogram was published in 2014 from a multi-institutional cohort of 514 patients from 13 Western and Eastern centers.[16] This nomogram included age, tumor size, number of lesions, nodal status, vascular invasion, and underlying cirrhotic liver parenchyma. Continuous variables for age and tumor size were included after cubic splines transformation, and the number of tumors was transformed to a binary variable (solitary vs multiple). Categorical variables were defined as follows: nodal status (pNx/pN0/pN1), vascular invasion (none/microvascular/macrovascular), and underlying cirrhotic liver (no/yes). The nomogram C-index in the derivation cohort was 0.69 (95% CI, 0.62−0.76), and bootstrap validation showed minimal evidence of overfit. The study did not include a validation cohort.

The Fudan score[15] was derived from an Asian single-center cohort (n = 344) in 2011, using only prognostic factors that are available preoperatively, such as alkaline phosphatase, CA 19-9, number of tumors, tumor size, and tumor boundary type. All variables were transformed into binary variables. Tumor boundary type was categorized on imaging as distinct vs obscure. Distinct boundary was defined as a regular border of thin ring-like iso-attenuation or arterial enhancement relative to the liver. Conversely, obscure boundary on imaging was described as an ill-defined border and represented a worse prognosis. These 5 prognostic factors resulted in a simple risk score in which 1 point was assigned for each factor.

Table 1. Descriptive Data in Memorial Sloan Kettering Cancer Center Cohort and Nomograms and Fudan Score Cohorts

Description	MSKCC (n = 188)	Hyder nomogram (n = 514)	Wang cohort (n = 367)	Fudan score (n = 344)
Age at surgery, y				
Median (range)	65.8 (19–89)		53 (23–78)	
Median (IQR)	65.7 (57.5–74.3)	59.2 (50–69)		
Older than 65 y, n (%)	96 (51)			87 (25.3)
Female sex, n (%)	110 (59.8)	241 (46.1)	121 (33)	145 (42.2)
Ethnicity, n (%)			NR	NR
White	135 (71.8)	314 (61.1)		
Black	9 (4.8)	16 (3.1)		
Asian	44 (23.4)	184 (35.8)		
Hepatitis, n (%)	18 (9.5)			
HBV	9 (4.8)	173 (33.4)	187 (51)	
HCV	9 (4.8)	19 (3.7)	8 (2.2)	96 (27.9)
Albumin, g/L		NR		
Median (range)	4.2 (2.1–6.3)		4.2 (3–6.5)	
<3.5 g/dL, n (%)	8 (4.2)			22 (6.4)
Total bilirubin, mg/L		NR		
Median (range)	0.6 (0.2–28.5)		0.8 (0.3–18.5)	
>17.1 mmol/L, n (%)	30 (15.9)			88 (25.6)
CA19-9, U/mL				
Median (range)	43 (1–53902)		41.2 (0.4–1000)	
Median (IQR)	43 (14.4–135.5)	25.9 (1–145)		
>37 U/mL, n (%)	66 (35.1)			200 (58.1)
CEA, μg/L				
Median (range)	2.3 (0.1–410)		2.5 (0.1–809.6)	
Median (IQR)	2.3 (1.3–3.4)	1.3 (0.3–3)		
>5 μg/L, n (%)	5 (2.7)			69 (23)
Tumor size, cm				
Median (range)	6 (1–24)		5.5 (0.4–22)	
Median (IQR)	6 (4–9)	6 (4–8.6)		
≥10, n (%)	39 (20.7)			56 (16.3)
Solitary lesion, n (%)	134 (71.3)	384 (74.7)		258 (75)
Multiple lesions, n (%)	54 (28.7)	130 (25.3)		86 (25)
2–3	19 (10.1)			
≥3	35 (18.6)		42 (11.4)	
<3	153 (81.4)		325 (88.6)	
Underlying liver, n (%)				NR
Steatosis	65 (35.9)	NR	NR	
Cirrhosis	9 (4.8)	44 (8.6)	78 (21.3)	
LVI, n (%)	68 (36.2)	124 (24.1)		57 (16.6)
Microvascular	46 (24.5)	68 (13.2)	54 (14.7)	
Macrovascular	22 (11.7)	56 (10.9)	37 (10.1)	
pN stage, n (%)				NR
pNx	96 (51.3)	262 (60)	74 (20.2)	
pN0	71 (37.6)	162 (31.5)	211 (57.5)	
pN1	21 (11.1)	90 (17.5)	82 (22.3)	
AJCC, 7th stage, n (%)		NR	NR	
I	75 (39.9)			129 (37.5)
II	49 (26)			75 (21.8)

(Continued)

Table 1. Continued

Description	MSKCC (n = 188)	Hyder nomogram (n = 514)	Wang cohort (n = 367)	Fudan score (n = 344)
III	31 (16.5)			23 (6.7)
IV	33 (17.6)			117 (34)
Extrahepatic invasion,* n (%)	36 (19.1)	14 (2.7)	35 (9.5)	NR
Morphological type, n (%)		NR		NR
Mass-forming	175 (93)		345 (94)	
Periductal invasion	13 (7)		20 (5.5)	
Adjuvant therapy, n (%)	51 (27.1)	122 (23.7)	NR	NR

*Gallbladder excluded.
CA 19-9, carcinogen antigen 19-9; GB, gallbladder; HBV, hepatitis B virus; HCV, hepatitis C virus; IQR, interquartile range; LVI, lymphovascular invasion; NR, not reported in the original publications; MSKCC, Memorial Sloan Kettering Cancer Center.

Patients were stratified in risk groups for death after resection: low risk for 0 points, intermediate risk for 1 point, high risk for 2–3 points, and extremely high risk for 4 to 5 points.

Statistical analysis

Overall survival and recurrence-free survival were calculated from the time of surgical resection until time of death (for OS), or until first relapse or death for progression-free survival. Recurrence was defined as tumor relapse, either biopsy proven or newly detected tumor on 2 consecutive radiologic images, with or without elevation of tumor markers. Patient who did not experience the event of interest by the end of the study were censored at the time of the last available follow-up. Overall survival and progression-free survival were estimated using the Kaplan-Meier method and compared between clinico-/pathologic characteristics using the log-rank test.

Performance of the nomograms was validated using the MSKCC patients in terms of discrimination and calibration. Discrimination was quantified with the C-index using Harrell's method.[19] The C-index provides the probability that, in a randomly selected pair of patients, in which one patient dies before the other, the patient who died first had the worse predicted outcomes from the nomogram. Calibration consisted of grouping patients in quartiles according to their nomogram-predicted probabilities, and comparing the mean of the group with the observed Kaplan-Meier OS curves. Results from calibration are presented as a calibration plot. To compare the 2 nomograms, a significance test was conducted using the bootstrap.[20] Specifically, bootstrap sample was drawn from our dataset and the C-index for both nomograms, as well as their difference, were estimated. The process was repeated 1,000 times and the differences obtained were ranked from smallest to largest. The p value is twice the rank of the observation nearest to 0 (representing no difference between the 2 nomograms).

All p values were based on 2-tailed statistical analysis and a p value <0.05 was considered to indicate statistical significance. All analyses were performed with SAS statistical software, version 9.2 (SAS Institute); SPSS software, version 22.0 for Windows (IBM SPSS); and R software, version 3.1.1.

RESULTS

Descriptive data

From January 1993 to May 2013, one hundred and ninety-nine consecutive patients underwent liver resection for ICC at MSKCC. Patients with mixed-type primary liver tumors (n = 5), distant metastatic disease at the time of resection (n = 1, peritoneal carcinosis), or postoperative death within 90 days after surgery (n = 5) were excluded. The remaining 188 patients were included in this study. The preoperative, operative, and pathologic characteristics are listed in Table 1 for both the MSKCC cohort and the 3 cohorts for whom the prognostic models are considered for validation. Patients from the MSKCC cohort were older (median age at surgery was 65.8 years), and were more often female (59.8%). The hepatitis B virus infection rate was lower in the MSKCC patient set. All cohorts were comparable in terms of preoperative biomarkers.

Liver resection consisted of a major hepatectomy (≥3 segments) in 125 patients (66.1%), achieving complete (R0) resection in 152 patients (80.4%). Extrahepatic resection was performed in 36 patients (19.1%) for direct invasion of diaphragm (n = 6), inferior vena cava (n = 4, with right adrenal gland resection in 3), stomach (n = 2), and hilar structures (n = 21, eg, biliary only, n = 14; vascular only, n = 2; combined biliary and vascular, n = 5). Lymph node dissection or sampling was performed in 92 patients (48.7%) and LN involvement was found in 21 patients (11.1%). Tumor size and number were comparable across the different studies, but vascular invasion and extrahepatic organ invasion were

Table 2. Variables Required for Nomograms, Fudan Score, and American Joint Committee on Cancer's Cancer Staging System, 7th Edition

Variable	Wang nomogram	Hyder nomogram	Fudan score	AJCC 7^{th} edition
Age		Continuous (transformed with cubic splines)		
Alkaline Phosphatase			Binary (≤147 U/L/>147 U/L)	
Biomarkers (CA1 9-9, CEA)	Continuous (linear)		CA19-9 only Binary (≤37 μg/L/>37 μg/L)	
Tumor size	Continuous (linear)	Continuous (transformed with cubic splines)	Binary <10 cm/≥10cm	
Tumor number	Ordinal variable Solitary/1−2/≥3	Binary solitary/multiple	Binary solitary/multiple	Binary variable solitary/multiple
Tumor boundary type			Binary distinct/obscure	
Lymphovascular invasion	Binary yes/no	Categorical variable micro/macro/no		Binary variable yes/no
Direct extrahepatic invasion	Binary variable yes/no			Binary variable yes/no
Lymph node status	Binary variable yes/no	Categorical variable pN0/pN1/pNx		Binary variable yes/no
Underlying cirrhosis		Binary variable yes/no		

CA 19-9, carcinogen antigen 19-9.

more frequently observed in the MSKCC cohort. Thirteen patients (6.9%) received neoadjuvant treatment for initial local unresectability, including systemic chemotherapy alone (n = 10) and intra-arterial floxuridine (n = 3) using a hepatic arterial infusion pump. Postoperatively, 51 patients (27.1%) received adjuvant chemotherapy, including gemcitabine-based therapy (n = 36, of which 4 receiving gemcitabine + oxaliplatine and 4 receiving gemcitabine + cisplatin), fluorouracil-based therapy (n = 9), and platinum compounds only (n = 1). Five patients received adjuvant intra-arterial floxuridine using a hepatic arterial infusion pump.

Survival data

With median follow-up of 42.5 months (range 5 to 192 months), we observed 98 deaths. The median OS was 47.8 months (95% CI, 37.6−68.9 months). The 1-, 3-, and 5-year OS rates were 91% (95% CI, 86−95%), 59% (95% CI, 51−67%), and 45% (95% CI, 37−53%), respectively. After primary resection, median recurrence-free survival was 21 months (95% CI, 11.8−30.1 months). Recurrence occurred in 110 patients (58.5%). Palliative systemic chemotherapy was offered after diagnosis of recurrence in 103 patients, combined with metastasectomy (n = 12), local ablation (n = 9), radiation therapy (n = 15), and liver-directed therapy (hepatic artery embolization or hepatic arterial infusion, n = 11).

Predictive performances

Variables required for both nomograms are summarized in Table 2. In the MSKCC cohort, 81 patients had missing values for the CEA and/or CA 19-9 levels. After excluding patients with missing tumor marker levels, the C-index for the nomogram from Wang and colleagues[7] was 0.72 (95% CI, 0.64-0.80) in this 107-patient cohort. The nomogram from Hyder and colleagues[16] had a C-index of 0.63 (95% CI, 0.57−0.69) in the whole cohort (n = 188) and 0.66 (95% CI, 0.56−0.74) in the cohort used for Wang nomogram assessment (n = 107). Nomogram calibration plots are displayed in Figure 1. Patient stratification using prediction tertiles was optimal with the Wang nomogram (p = 0.003) and the Hyder nomogram (p = 0.021) (Fig. 2). Different 5-year OS prediction ranges in each tertile corresponded to distinct median OS observed in our cohort (n = 107). Stratification appeared more distinct between worse-risk patients (first and second tertiles) with the Wang nomogram. Figure 3 shows how inconsistent the survival predictions from both nomograms are in some patients.

The AJCC Cancer Staging System (7^{th} ed) had a C-index of 0.63 (95% CI, 0.58−0.67). Survival in patients stratified by AJCC stages is represented in Figure 2. As

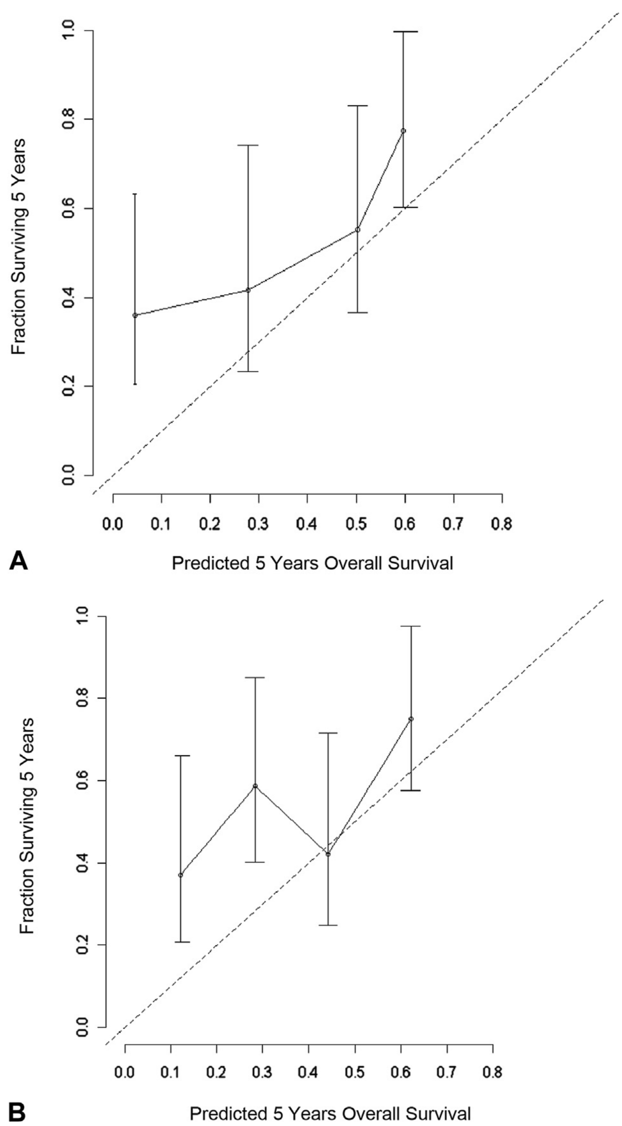


Figure 1. Calibration plots of the (A) Wang nomogram and (B) the Hyder nomogram in 107 patients. The dashed line represents the reference line.

shown in Figure 4A, tumor stage according to the AJCC was a significant predictive factor for OS in the whole cohort ($p < 0.001$), but separation of the curves was poor (median OS for stage II was 32.7 months, median OS for stage III patients was 51.9 months, and median OS for stage IV was 25.2 months). Excluding patients with unknown nodal status (pNx) improved predictive accuracy (C-index = 0.68; 95% CI, 0.61−0.75). Still, patients stratification remained poor, with unclear survival differences among stage II, III, and IV patients, as displayed in Figure 4B (median OS for stage II was 32.9 months, median OS for stage III patients was 28 months, and median OS for stage IV was 26.9 months).

One hundred and thirteen patients had available data for Fudan score assessment (75 patients had missing values for the alkaline phosphatase and/or CA 19-9 levels). Discrimination was acceptable (C-index = 0.55; 95% CI, 0.47−0.64) and prognostic stratification tended to be significant ($p = 0.051$), but stratification with respect to the different risk score strata was not optimal (Fig. 4C).

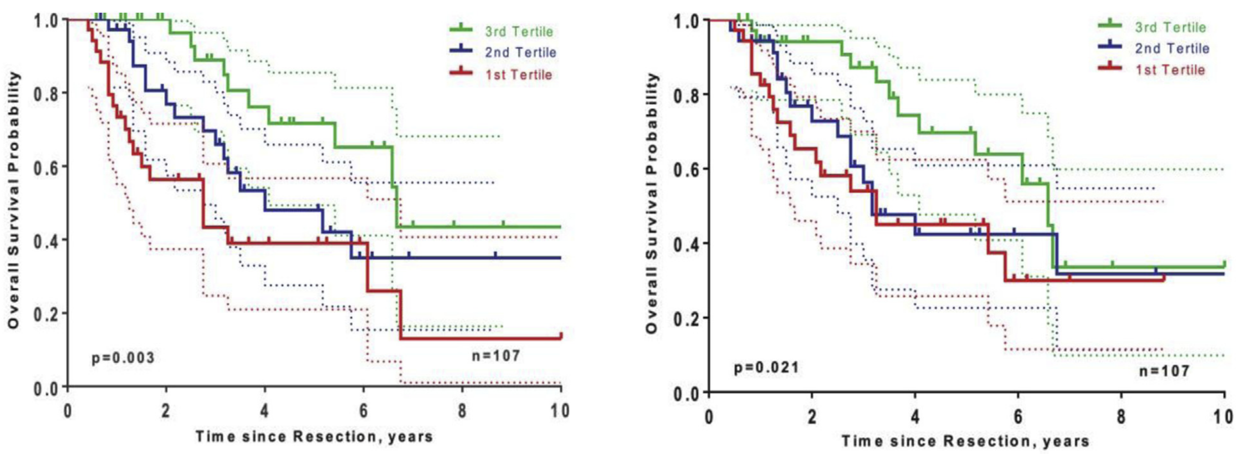
Impact of adjuvant therapy

Patients who received adjuvant therapy were mostly those with a predicted poor prognosis by both nomograms (Wang first tertile, 59.4%; $p < 0.001$; Hyder first tertile, 43.8%; $p = 0.17$) or with more advanced disease (AJCC stage III to IV, 51%; $p = 0.001$; high-risk or extremely high-risk Fudan score, 57.6%; $p = 0.001$). Overall, 51 patients (27.1%) received adjuvant therapy, which was a negative prognostic variable in univariate analysis (hazard ratio = 5.728; $p = 0.017$).

DISCUSSION

This study is the first to externally validate 2 recently published nomograms and 1 risk score for outcomes after resection of ICC. Both nomograms showed an optimal discrimination and calibration and provided an accurate patient stratification. In contrast, the Fudan score failed to achieve optimal discrimination and correct stratification in the current study.

Both nomograms included different variables. Notably, the Wang nomogram included the tumor markers CA 19-9 and CEA. Although those biomarkers are not considered in other models or in the AJCC staging system, they have been reported as important prognostic variables previously.[21,22] Additionally, direct extrahepatic invasion, already included in the AJCC staging system, was included in the Wang nomogram and might reflect adverse tumor biology. Conversely, the Hyder nomogram did not include either of these variables but incorporated age and underlying cirrhosis as prognostic factors. Although underlying cirrhosis has been reported to be a poor prognostic factor in ICC, no studies other than the Hyder nomogram have related it to OS after resection of ICC.[3] In our cohort, the underlying cirrhosis rate was low (4.8%), resulting in a low additive predictive capacity. Another noteworthy difference between both nomograms is related to LN status. Hyder and colleagues[16] coded this variable as a categorical variable (pN0, pN1, or pNx), and patients with missing LN status (Nx) were considered node negative (N0) in the Wang nomogram. Some studies have suggested that no suspicion of nodal disease based on preoperative imaging and intraoperative assessment can act as a reasonable surrogate to routine LN dissection and reported similar



	Predicted 5-year OS range	Observed median OS, years
First Tertile	0%-22%	2.7 (1.2-4.3)
Second Tertile	23%-52%	4 (1.7-6.4)
Third Tertile	53%-65%	6.7 (5.1-8.3)

A

	Predicted 5-year OS range	Observed median OS, years
First Tertile	1%-24%	3.3 (1.7-4.8)
Second Tertile	25%-44%	3.2 (1.8-4.6)
Third Tertile	45%-83%	6.6 (5.4-7.8)

B

Figure 2. Kaplan-Meier overall survival (OS) curves for patients stratified by predicted tertiles by the (A) Wang nomogram and the (B) Hyder nomogram.

outcomes in ICC patients with pN0 and pNx disease.[23,24] However, portal LN involvement is an independent prognostic factor in ICC, and such a nodal status classification might be inappropriate, as several studies advocate a routine lymphadenectomy for accurate staging.[25-27] Other variables were included in both nomograms but were modeled differently. Tumor size had been removed from the T stage in the 7th edition of the AJCC staging system, but was included as a variable in both the Wang and Hyder nomograms, modeled as a continuous variable in the former. Estimating the clinical meaning of continuous variables using a linear model might be inaccurate. In contrast, Hyder and colleagues[16] observed in their study that the actual effect of tumor size on the risk of death was linear up to nearly 7 cm, but this effect leveled off when >7 cm; and tumor size was transformed in a nonlinear continuous variable using restricted cubic splines in this model. The number of liver lesions was also modeled differently for both nomograms. The multiplicity of lesions appears to represent strong evidence of unfavorable tumor biology. Wang and colleagues[7] considered the number of lesions as an ordinal variable, stratifying patients as solitary tumor, multiple tumors but <3, or ≥3. Although multiplicity of lesions might represent aggressive tumor biology, such an impact on OS and differential tumor number has never been reported. Similarly, vascular invasion is a poor prognostic factor and a recurrence risk factor, and differentiating microvascular and macrovascular invasions has been suggested as an important distinction in staging ICC, which was only considered in the Hyder nomogram.[28] Taken altogether, these differences might account for the inconsistency in 5-year OS prediction between both nomograms (Fig. 3).

Despite these discrepancies, both nomograms provided optimal discrimination and stratification when compared on the same patient population. Patients' stratification using predicted 5-year OS tertiles appeared slightly more accurate with the Wang nomogram (Figure 2). The C-index tended to show a more accurate discrimination with the Wang nomogram (C-index = 0.72; 95% CI, 0.64−0.80) than with the Hyder nomogram (C-index = 0.66; 95% CI, 0.56−0.74). This difference was not significant statistically (p = 0.17), which is not surprising, given that large sample sizes are typically required to establish statistical significance between C-indices. Accordingly, although one cannot formally endorse the Wang nomogram for use in clinical practice, it is interesting to note that this prognostic model was developed in an Eastern population but fit well in our Western cohort, providing the most clear-cut patient stratification in distinct survival groups. The Wang nomogram requires both routine preoperative CA 19-9 and CEA levels and routine LN dissection to be applicable. In contrast, the Hyder nomogram might have a broader clinical applicability by obviating routine preoperative tumor marker assessment and routine formal

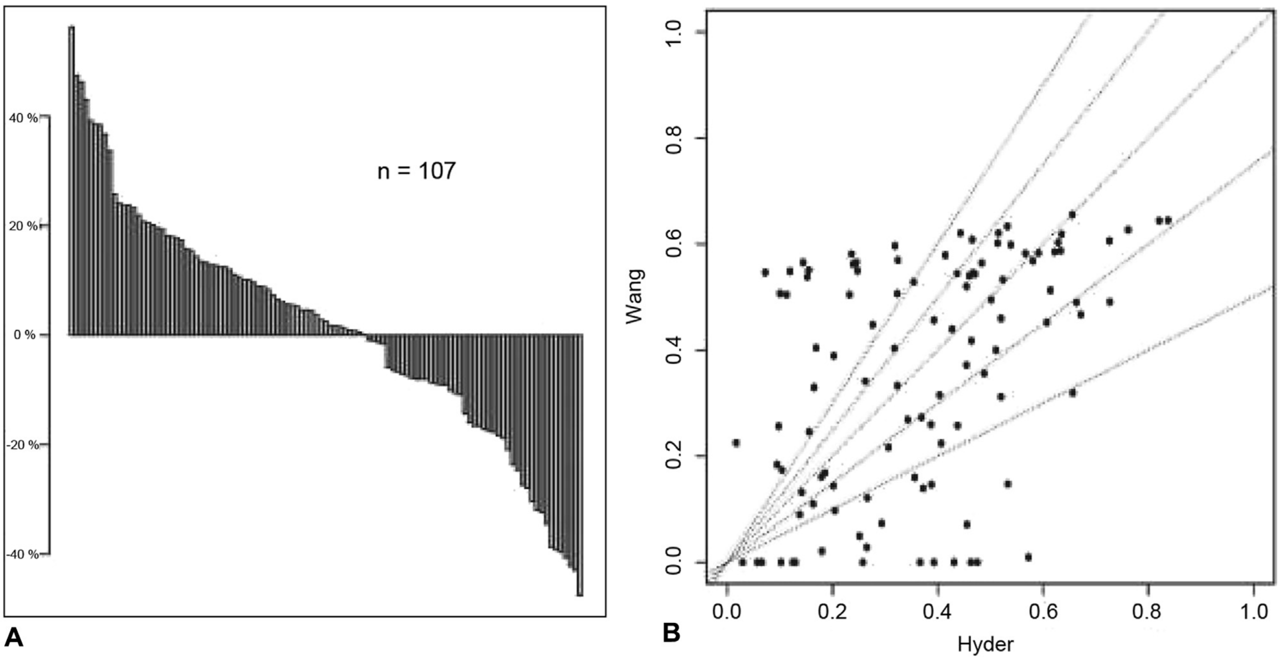


Figure 3. Differences in 5-year overall survival (OS) probability for each patient by Hyder and Wang nomograms. (A) The waterfall plot shows the inconsistency between both nomograms (Hyder and Wang). Patients with a difference in 5-year OS probability >0% had better predicted 5-year OS probability by Hyder nomogram. Patients with a difference in 5-year OS probability <0% had better predicted 5-year OS probability by Wang nomogram. This inconsistency between both nomograms is completely random, without any correlation, as shown on the (B) cloud plot.

portal lymphadenectomy and being developed from an international cohort.

The Fudan score provided moderate but acceptable discrimination (C-index >0.5).[19] However, stratification using the 4 risk levels of this score was not optimal (Fig. 4C). These findings are likely the result of several factors. First, tumor boundary type is a very subjective variable, resulting in substantial inter-observer variability. Second, preoperative evaluation of the number of tumors might be inaccurate, given that detecting small satellite nodules on preoperative imaging can be challenging.[17] Additionally, serum alkaline phosphatase level was a binary variable in this score, but that has never been reported as a prognostic factor in ICC. In addition, serum alkaline phosphatase activity is increased in many pathologic conditions, associated with an increased all-cause mortality risk, and reported to have different baseline levels between Eastern and Western populations.[29] Using this variable might limit the applicability of this score developed in an Eastern population to a Western population. Unlike the AJCC staging system and both nomograms that are pathology-based prognostic tools, the Fudan score allows a preoperative prognostic estimation. Such prognostic tools can help to identify preoperatively patients with poor prognosis and improve their management.

The AJCC staging system provided a correct discrimination (0.63; 95% CI, 0.58−0.67), but a suboptimal stratification with a worse median OS for stage II patients (32.7 months) than for stage III patients (51.9 months). The AJCC staging system discrimination was improved when the staging system was applied only to the 92 patients who underwent LN dissection (C-index = 0.68; 95% CI, 0.61−0.75). Similarly, patient stratification was slightly more accurate with median OS in stage II and III of 32.9 and 28 months, respectively, but curves separation remained poor (Figs. 4A, B). Consequently, the AJCC Cancer Staging System might not be appropriate for prognostic estimations in patients without LN dissection. In addition, the lack of stratification between stages II and III might be due to the definition of pathologic T stage. Stage II encompasses pT2 tumors (T2a: solitary tumor with vascular invasion and T2b: multiple tumors with or without vascular invasion) and stage III defines tumor perforating the visceral peritoneum or directly involving the local extrahepatic structures. The clinical significance of multiple tumors (pT2b), meaning intrahepatic metastases or satellite lesions, is likely underestimated by this definition and would explain the overlapping survival curves of stage II patients (pT2b), who had inferior survival compared with stage III patients with solitary tumors, regardless of size.

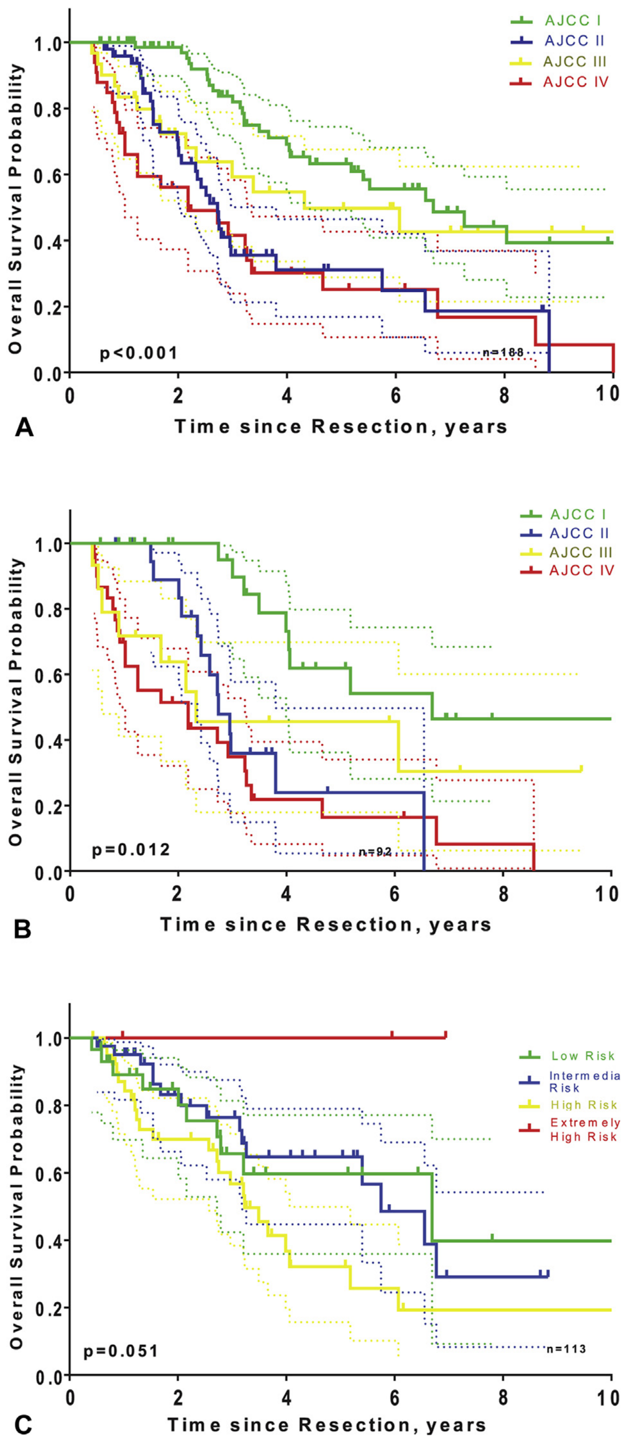


Figure 4. Kaplan-Meier overall survival curves for patients stratified according to the AJCC Staging System (7th ed) (A) in the Memorial Sloan Kettering Cancer Center cohort, (B) in only patients with pathologic lymph node status, and (C) according to the Fudan score.

Adjuvant therapy is usually recommended for patient with worrisome tumor features (eg, positive margin, node-positive disease, and vascular invasion) and its positive impact on prognosis is based on findings from randomized trials in nonresected patients with advanced or metastatic biliary neoplasms and retrospective series.[9,10,30] In the current study, adjuvant therapy was delivered to high-risk patients and did not modify patient stratification significantly. Survival was worse in patients receiving adjuvant therapy in the whole cohort and in stratified patient's subsets. This finding, however, should be considered carefully, given the small proportion of patients receiving adjuvant therapy (27.1% in the whole cohort and 30.3% in the 107 patients used for nomograms validation).

The current study has several limitations. First, missing data on tumor markers reduced considerably the number of patients available for validation of the Wang and colleagues[7] nomogram. Second, the validation cohort was similar to the derivation cohorts of the published nomograms with regard to key prognostic factors identified in a recent meta-analysis (Fig. 1), but for the underlying liver disease potentially driving tumor biology, a large proportion of patients in the current study had nonalcoholic steatosis in the nontumorous liver, much higher than reported previously in Western studies.[31-33] Similarly, hepatitis B virus (4.8%) and cirrhosis (4.8%) rates were lower in the current study. Additionally, in the current cohort, patients who underwent resection after neoadjuvant therapy (n = 13) might represent a patient category not appropriate for these prognostic models.

CONCLUSIONS

Both nomograms can be useful for patient prognosis estimation and recommendation for adjuvant therapy after liver resection for ICC. The nomogram proposed by Wang and colleagues[7] appeared to have the best overall prognostic accuracy in the present cohort, but requires routine portal LN dissection and preoperative tumor markers (CEA and CA 19-9), in addition to the variables necessary for the AJCC staging system, for optimal performance. By contrast, the Fudan score and AJCC staging system were of limited utility in this dataset. Additional investigations are needed in wider series to define the most appropriate prognostic nomogram for clinical practice.

Author Contributions

Study conception and design: Doussot, Groot-Koerkamp, DeMatteo, Allen, Kingham, D'Angelica, Jarnagin

Acquisition of data: Doussot, Groot-Koerkamp, Wiggers
Analysis and interpretation of data: Doussot, Groot-Koerkamp, Wiggers, Chou, Gonen
Drafting of manuscript: Doussot, Groot-Koerkamp, Wiggers, Chou, Gonen, Jarnagin
Critical revision: Doussot, Groot-Koerkamp, Wiggers, Chou, Gonen, DeMatteo, Allen, Kingham, D'Angelica, Jarnagin

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Annexe 3.

Recurrence Patterns and Disease-Free Survival after Resection of Intrahepatic Cholangiocarcinoma: Preoperative and Postoperative Prognostic Models

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BACKGROUND: Liver resection is the most effective treatment for intrahepatic cholangiocarcinoma. Recurrent disease is frequent; however, recurrence patterns are ill-defined and prognostic models are lacking.

STUDY DESIGN: A primary cohort of 189 patients who underwent resection for intrahepatic cholangiocarcinoma was used for recurrence patterns analysis within and after 24 months. Based on independent factors for disease-free survival identified in Cox regression analysis, preoperative and postoperative models were developed using a recursive partitioning method. Models were externally validated using a multicenter cohort of 522 resected patients (Association Française de Chirurgie intrahepatic cholangiocarcinoma study group).

RESULTS: Recurrence within 24 months most often involved the liver (82.7%), and most recurrences after 24 months were strictly extrahepatic (61.1%). In multivariable analysis of the primary cohort, independent preoperative factors for disease-free survival were tumor size and multifocality (based on imaging); tumor size, multifocality, vascular invasion, and lymph node metastases (based on pathology) were independent postoperative factors. The preoperative model allowed patient classification into low-risk and high-risk groups for recurrence. In the validation cohort ($n = 522$), high-risk patients had a greater likelihood of recurrence (hazard ratio = 2.17; 95% CI, 1.74–2.72; $p < 0.001$). The postoperative model included tumor size, vascular invasion, and positive nodal disease on pathology and classified patients in low-, intermediate-, and high-risk groups in the primary cohort. As compared with low-risk patients in the validation cohort, intermediate- and high-risk patients were more likely to experience recurrence (hazard ratio = 1.9; 95% CI, 1.41–2.47; $p < 0.001$ and hazard ratio = 2.99; 95% CI, 2.08–4.31; $p < 0.001$, respectively).

CONCLUSIONS: Recurrence patterns are time dependent. Both models as developed and validated in this study classified patients in distinct recurrence risk groups, which can guide treatment recommendations. (J Am Coll Surg 2016;223:493–505. © 2016 by the American College of Surgeons. Published by Elsevier Inc. All rights reserved.)

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Abbreviations and Acronyms

DFS	= disease-free survival
HAI-FUDR	= hepatic arterial infusion with floxuridine
HR	= hazard ratio
IHCC	= intrahepatic cholangiocarcinoma
OS	= overall survival

Intrahepatic cholangiocarcinoma (IHCC) incidence has risen during the last 3 decades.[1,2] To date, the only potentially curative treatment is complete resection, which offers 5-year overall survival (OS) rates ranging from 21% to 35% and a median OS up to 39 months.[3-6] According to the National Comprehensive Cancer Network guidelines, adjuvant therapy is recommended mainly in patients at risk for recurrence[7] because postoperative recurrence rates range from 53% to 79%, and most patients eventually die of disease.[6-10] The most frequent site of failure is the liver, either alone (range 60.9% to 62.7%) or associated with extrahepatic recurrence (18.6%); extrahepatic-only recurrence is less common (21%).[8,9] Understanding of recurrence patterns could help to better appraise the recurrence risk, to tailor postoperative monitoring, and to guide perioperative treatment strategies, especially as locoregional therapies for IHCC are emerging.[11-14] Additionally, some patients recurring early and ultimately dying shortly after resection likely do not benefit from surgery alone, and identification of these patients at presentation could optimize their management.

Although evidence supporting the use of perioperative chemotherapy vs surgery alone for resectable IHCC is lacking, several studies reported promising results in initially unresectable patients who experienced significant tumor reduction and conversion to resection after preoperative systemic or hepatic intraarterial chemotherapy.[15-17] Based on these data, high-risk resectable patients might benefit from a multimodal approach involving systemic and/or liver-directed therapy.

The current study sought to identify patients at greatest risk for early recurrence by exploring the predictive factors associated with recurrence patterns and disease-free survival and developing a recurrence risk model.

METHODS

Patients and study design

A retrospective study was conducted on a cohort of patients who underwent curative-intent hepatectomy from January 1993 to May 2013 for IHCC at Memorial Sloan Kettering Cancer Center. Data were collected from a prospectively maintained liver resection database. Patients were deemed resectable according to the following criteria: R0 resection potentially achievable; adequate future liver remnant function and volume (minimum of 2 contiguous liver segments), with adequate perfusion and venous and biliary drainage; and general health conditions suitable with liver surgery. The authors' approach to intraoperative and perioperative management has been published previously.[8,18] Exclusion criteria included a diagnosis of mixed cholangiocarcinoma and hepatocellular carcinoma and a palliative-intent resection, such as R2 resection. Additionally, patients deceased within 90 days after surgery were excluded from the outcomes analyses.[19] The IRB approved this study.

A distinct cohort of patients who underwent curative-intent partial hepatectomy for IHCC was retrospectively analyzed and formed the validation cohort of this study. Briefly, data from all consecutive patients submitted to curative-intent resection for IHCC from January 1989 to March 2009 at 24 tertiary hepatobiliary centers were collected from a dedicated multi-institutional database related to studies from the Association Française de Chirurgie IHCC study group published previously.[4,20] Authorization from the Association Française de Chirurgie was obtained for using these data. Inclusion and exclusion criteria for the current study were those already mentioned.

Data collection

Clinical preoperative variables included demographics and preoperative tumor markers (eg carcinogen antigen 19-9). Preoperative tumor features based on imaging, including CT, MRI, ultrasonography, and PET scan, were documented. Operative data were also collected. Liver resection of 3 or more segments was defined as major resection. In both cohorts, resections were extended to extrahepatic structures when required to achieve a macroscopically complete resection. Lymphadenectomy was performed at the discretion of the surgeon, either as a formal peripancreatic and portocaval lymph node dissection or as a targeted excision according to preoperative imaging and intraoperative findings.

Pathology data

Pathologic variables included size and number of tumors, differentiation grade, resection margin status, vascular invasion, perineural invasion, nodal status, and histology of the non-tumoral liver parenchyma. Extrahepatic invasion was defined as direct invasion of any extrahepatic organs, excluding the gallbladder (pT3). Morphologic subtype was defined as mass-forming, periductal infiltrating, intraductal growth, and mixed.[21,22] Tumor staging was determined using the seventh edition of the *American Joint Committee on Cancer Staging System*.[23]

Follow-up and recurrence data

Clinical and radiographic monitoring was performed every 4 to 6 months. Adjuvant therapy was offered at the discretion of the multidisciplinary team, primarily to patients considered at high risk for recurrence. Recurrence was defined as any sign of recurrent cholangiocarcinoma, either biopsy-proven or suspected on cross-sectional imaging (with documented progression on serial imaging) with or without elevated carcinogen antigen 19-9 level. In the primary cohort, initial recurrence site was categorized as hepatic only or extrahepatic or synchronous hepatic and distant recurrence. Recurrence treatment initiation date and treatment modalities were documented. Multimodal therapy was defined as recurrence management involving systemic chemotherapy associated with liver-directed therapy.

Due to missing data, recurrence site and management were not fully documented in the validation cohort. Consequently, recurrence patterns could be assessed in the primary cohort only.

Study objectives

The first aim of this study was to develop and validate prognostic models of recurrence based on independent prognostic factors for disease-free survival (DFS). Although OS remains the standard end point in survival analysis, DFS stands as a relevant end point in the setting of IHCC. Recurrence after curative-intent hepatectomy is frequently observed and patients eventually die of their recurrent disease. However, early and multimodal management of the recurrence is reported to be associated with prolonged survival.[9] Recurrence-specific prognostic models might be helpful for identifying patients at high risk for recurrence, helping with perioperative decision making and improving early recurrence detection and management.

The second objective was to define recurrence patterns. Although recurrence might be observed long after resection, Spolverato and colleagues[24] recently reported that recurrences are generally observed within 5 years, with the highest risk being within the 24 months after surgery. Additionally, median DFS does not exceed 24 months (range from 20 to 26 months) in the current literature.[8,9,24,25] Therefore, patterns of recurrence were assessed based on occurrence within or after 24 months of resection.

Statistical analysis

Categorical variables were summarized using percentages, and continuous variables were summarized using mean (SD) or median (range), as appropriate. Characteristics of patients were compared using the chi-square test for categorical variables and the *t*-test or the Mann-Whitney U test for continuous variables, as appropriate. Overall survival and DFS were estimated using the Kaplan-Meier method and corresponded to the interval between primary resection date and the date of last follow-up and recurrence date, respectively. Patients who were dead or with recurrence at last follow-up were considered as event, and patients who were alive and disease-free at last follow-up were censored for DFS analysis. In turn, patients who were dead at last follow-up were considered as event, and patients who were alive at last follow-up were censored for OS analysis. Differences in terms of DFS between groups were compared using the log-rank test. Variables in the univariate analysis with $p < 0.1$ were included in a Cox proportional hazard model to identify independent significant prognostic factors. Backward selection was used with a 0.1 cutoff for entry into the model. The first model included preoperative data only and the second included postoperative histopathologic data derived from the resected specimen.

In addition, based on the independent predictors for DFS in either preoperative and postoperative model, patients were classified into preoperative and postoperative risk groups of recurrence using a recursive partitioning method.[26,27] Briefly, a recursive partitioning consists of creating a decision tree that strives to correctly classify members of the population based on several dichotomous independent variables. Performance of both preoperative and postoperative models was validated using the validation cohort in terms of stratification of recurrence rate and DFS. All p values were based on 2-tailed statistical analysis and a p value <0.05 was considered to indicate statistical significance. All analyses were performed with SPSS software, version 22.0 for Windows (SPSS Inc.) and R software, version 3.1.1.

RESULTS

Perioperative data in primary and validation cohorts

During the study period, 200 consecutive patients underwent liver resection for IHCC at Memorial Sloan Kettering Cancer Center. Patients with mixed-type primary liver tumors ($n = 5$), distant metastatic disease at the time of resection ($n = 1$), or postoperative death within 90 days after surgery ($n = 5$) were excluded. The remaining 189 patients were included in the analysis as the primary cohort. For the validation cohort, 522 patients with curative-intent resection were included. Preoperative, operative, and pathologic characteristics in the primary and validation cohorts are listed in Table 1. There were

Table 1. Clinicopathologic Features in the Primary (Memorial Sloan Kettering Cancer Center) and Validation (Association Française de Chirurgie) Cohorts of Patients Resected for Intrahepatic Cholangiocarcinoma

Variable	Memorial Sloan Kettering Cancer Center cohort (n = 189)	Association Française de Chirurgie cohort (n = 522)	p Value
Preoperative			
Age at surgery, y, mean (SD)	65.4 (11.8)	64 (11.7)	0.35
Female, n (%)	114 (60.3)	268 (51.3)	0.04
Hepatitis, n (%)	18 (9.5)	32 (6.1)	0.14
Hepatitis B virus	9 (4.8)	NA	
Hepatitis C virus	9 (4.8)	NA	
PSC/IBD, n (%)	7 (3.7)	NA	
Imaging modality, n (%)			
CT	170 (89.9)	NA	
MRI	114 (60.3)	NA	
Ultrasound	70 (37)	NA	
PET	59 (31.2)	NA	
Preoperative tumor size, cm, mean (SD)	6.5 (3.6)	6.8 (3.8)	0.16
Preoperative multiple tumor, n (%)	33 (17.5)	79 (15.1)	0.49
Preoperative enlarged lymph node, n (%)	16 (8.5)	NA	
Total bilirubin, mg/L, mean (SD)	1.2 (3.1)	1.55 (3.4)	<0.001
Carcinogen antigen 19-9, U/mL, mean (SD)	1,847.7 (5,354.1)	1,547 (7,101)	0.001
Neoadjuvant therapy, n (%)	10 (5.3)	34 (6.5)	0.6
Postoperative			
Major resection, n (%)	124 (65.6)	401 (76.8)	0.004
Tumor size, cm, mean (SD)	6.9 (3.9)	7.1 (4)	0.9
Multiple lesions, n (%)	54 (28.6)	187 (35.8)	0.08
Underlying liver, n (%)			0.053
Steatosis	69 (36.5)	142 (27.2)	
Cirrhosis	9 (4.8)	25 (4.8)	
Vascular invasion, n (%)			0.6
Absent	121 (64)	321 (61.5)	
Present	68 (36)	201 (38.5)	
Microvascular	46 (24.3)	NA	
Macrovascular	22 (11.6)	NA	
Perineural invasion, n (%)	54 (28.6)	124 (23.8)	0.21
Extrahepatic invasion, n (%)*	22 (11.6)	34 (6.5)	0.012
Morphologic subtype, n (%)			<0.001
Mass-forming	176 (93.1)	367 (70.3)	
Periductal invasion	13 (6.9)	9 (1.7)	
Intraductal growth	—	6 (1.1)	
Mixed subtype	—	58 (11.1)	
Unknown	—	82 (15.7)	
Margin status, n (%)			0.006
Negative	152 (80.4)	365 (69.9)	
Positive	37 (19.6)	157 (30.1)	
pN stage, n (%)			0.22
pNx	97 (51.3)	246 (47.1)	
pN0	71 (37.6)	191 (36.6)	
pN1	21 (11.1)	85 (16.3)	
Adjuvant therapy, n (%)	51 (27)	178 (34.1)	0.084

*Gallbladder excluded.
IBD, inflammatory bowel disease; PSC, primary sclerosing cholangitis.

significant differences in terms of sex; total bilirubin and carcinogen antigen 19-9 levels; and extent of resection and tumor features, such as extrahepatic invasion rate, morphologic subtypes, and resection margin status between the primary and the validation cohorts.

Survival data, recurrence patterns, and management

In the primary cohort, median OS was 47.8 months (95% CI, 30.3−65.4 months) (Fig. 1A). After primary resection, median DFS was 23.1 months (95% CI, 14.6−31.6 months). After a median follow-up of 42.5 months (range 5 to 192 months), recurrence was documented in 110 patients (58.2%). Fifty-six patients (50.9%) experienced recurrence confined to the liver. Extrahepatic recurrences were strictly extrahepatic in 27 patients and simultaneously involving the liver in 27 patients. Recurrence rate within 24 months was 83.6% (n = 92) and 18 patients eventually recurred after 24 months, at a median follow-up time of 64.3 months (range 26 to 192 months). Recurrence patterns were significantly different between the 2 groups ($p < 0.001$) (Fig. 1B). Hepatic recurrence, whether confined to the liver or associated with distant recurrence (n = 83), overwhelmingly occurred in patients who recurred within 24 months (n = 76 [91.6%]). In this group, the liver was involved in 82.7% of patients compared with 38.9% in patients who recurred after 24 months. In patients who failed after 24 months (n = 18), 11 (61.1%) recurred distantly (lung, n = 6; retroperitoneal nodes, n = 2; bone, n = 2; ovarian, n = 1). Recurrence rate and patterns did not differ over time.

Of note, among patients treated with neoadjuvant therapy (n = 10), 8 patients (80%) experienced recurrence, all of which were within 24 months after resection and were extrahepatic only in 4 patients. As shown in Figure 2, recurrence treatment modalities were different across the DFS groups ($p = 0.033$). Two-thirds of patients who recurred at 24 or more months received multimodal therapy. Surgical resection was performed in 20 patients (liver, n = 10; lung, n = 6; bone, n = 3, ovary, n = 1). Metastasis ablation was exclusively performed for recurrent disease isolated to the liver (n = 11; radiofrequency ablation, n = 9; microwave ablation, n = 2) and was combined with liver-directed therapy in 5 patients (hepatic arterial infusion with floxuridine [HAI-FUDR], n = 3; hepatic artery embolization, n = 2). Overall, systemic chemotherapy was used in 92 patients and consisted of gemcitabine-based regimen in 60 patients (65.2%). Median OS after recurrence treatment initiation was 19 months (95% CI, 14.1−23.9) and was prolonged significantly in patients managed with multimodal therapy ($p < 0.001$).

In the validation cohort, median OS was 49 months (95% CI, 41−56.9 months). After primary resection, median DFS was 18 months (95% CI, 16.6−19.4 months). After a median follow-up of 35 months (range 3 to 211 months), recurrence was documented in 248 patients (47.5%). Recurrence rate within 24 months was 89.9% (n = 223) and 25 patients eventually recurred after 24 months, at a median follow-up time of 35 months (range 25 to 101 months).

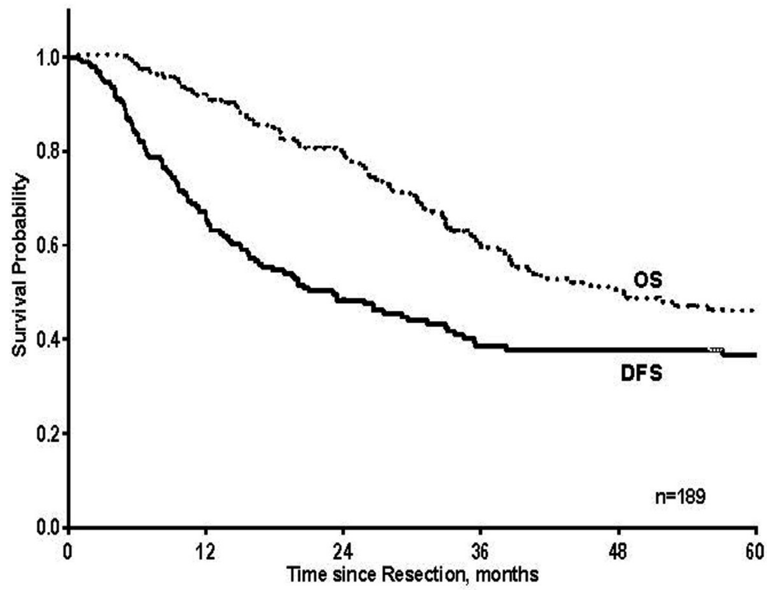
Prognostic factors for disease-free survival in the primary cohort

The full cohort (n = 189) was included in DFS analyses. Univariable and multivariable analysis for DFS are shown in Table 2. Preoperative tumor size (hazard ratio [HR] = 1.09; 95% CI, 1.04−1.14; $p < 0.001$) and multifocality on imaging (HR = 1.73; 95% CI, 1.12−2.70; $p = 0.013$) were independently associated with a shorter DFS. Regarding postoperative factors, tumor size (HR = 1.10; 95% CI, 1.05−1.15; $p < 0.001$), multifocality (HR = 1.82; 95% CI, 1.22−2.71; $p = 0.003$), and vascular invasion and positive nodal disease (HR = 2.77; 95% CI, 1.52−5.03; $p < 0.001$) on pathology were independent factors of shorter DFS.

Development of recurrence risk models on the primary cohort

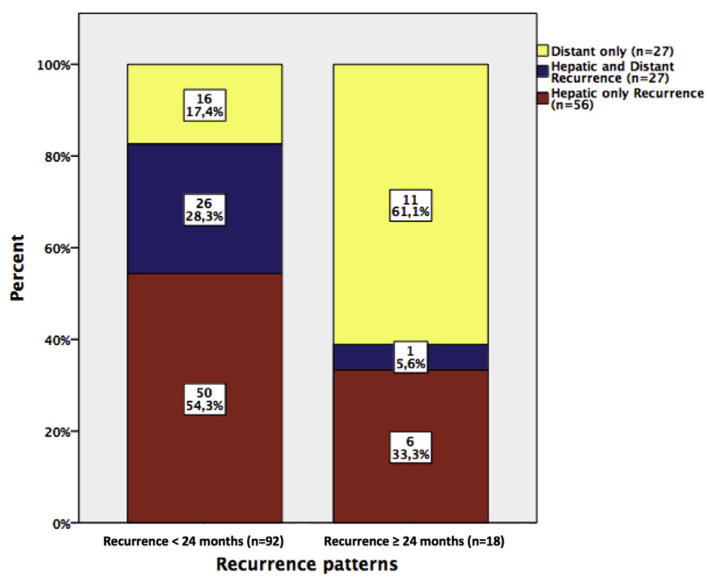
Using a recursive partitioning method, preoperative and postoperative independent factors for DFS, as mentioned, were used for developing preoperative and postoperative recurrence risk models, respectively. Patient subsets with low and high recurrence risk were then identified using the preoperative model (classification tree, Fig. 3A). Tumor size was the most important variable and multifocal disease helped to further separate patients in low- and high-risk groups into the preoperative model. Patients preoperatively classified as low risk had a significantly longer DFS than patients classified as at high risk for recurrence (median DFS 31.3 months vs 12 months; $p < 0.001$, Fig. 3B). Recurrence patterns observed in the full primary cohort remained comparable between the 2 groups, with recurrence mostly involving the liver within 24 months, and later recurrences were mostly isolated to an extrahepatic site (eTable 1, available online).

In contrast, 3 risk subsets were identified in the postoperative model (Fig. 4A; low, intermediate, and high). Nodal status was the most important variable, and multifocal disease was replaced in the postoperative model by vascular invasion for further stratifying patients with node-negative tumor <6 cm. In the full primary cohort



Patients at risk (n=189)	**153**	**119**	**79**	**57**	**31**
Patients at risk (n=189)	**112**	**69**	**45**	**37**	**21**

A



B

Figure 1. Kaplan-Meier survival curves for (A) all patients included (n = 189) and (B) recurrence patterns for patients categorized by their disease-free survival (DFS). Fifty-two patients have not recurred at last follow-up. Dotted line, overall survival (OS) curve; black line, DFS curve. (In each group, the proportion of patients experiencing each recurrence patterns is labeled on each corresponding bar.)

(n = 189), patients with pNx status (n = 97) were considered as pN0. Median DFS differed significantly between risk groups (low risk, 48 months; intermediate risk, 18 months; high risk, 9 months; $p < 0.001$, Fig. 4B). Similarly, the time dependence of recurrence patterns was again observed across these 3 groups (eTable 1, available

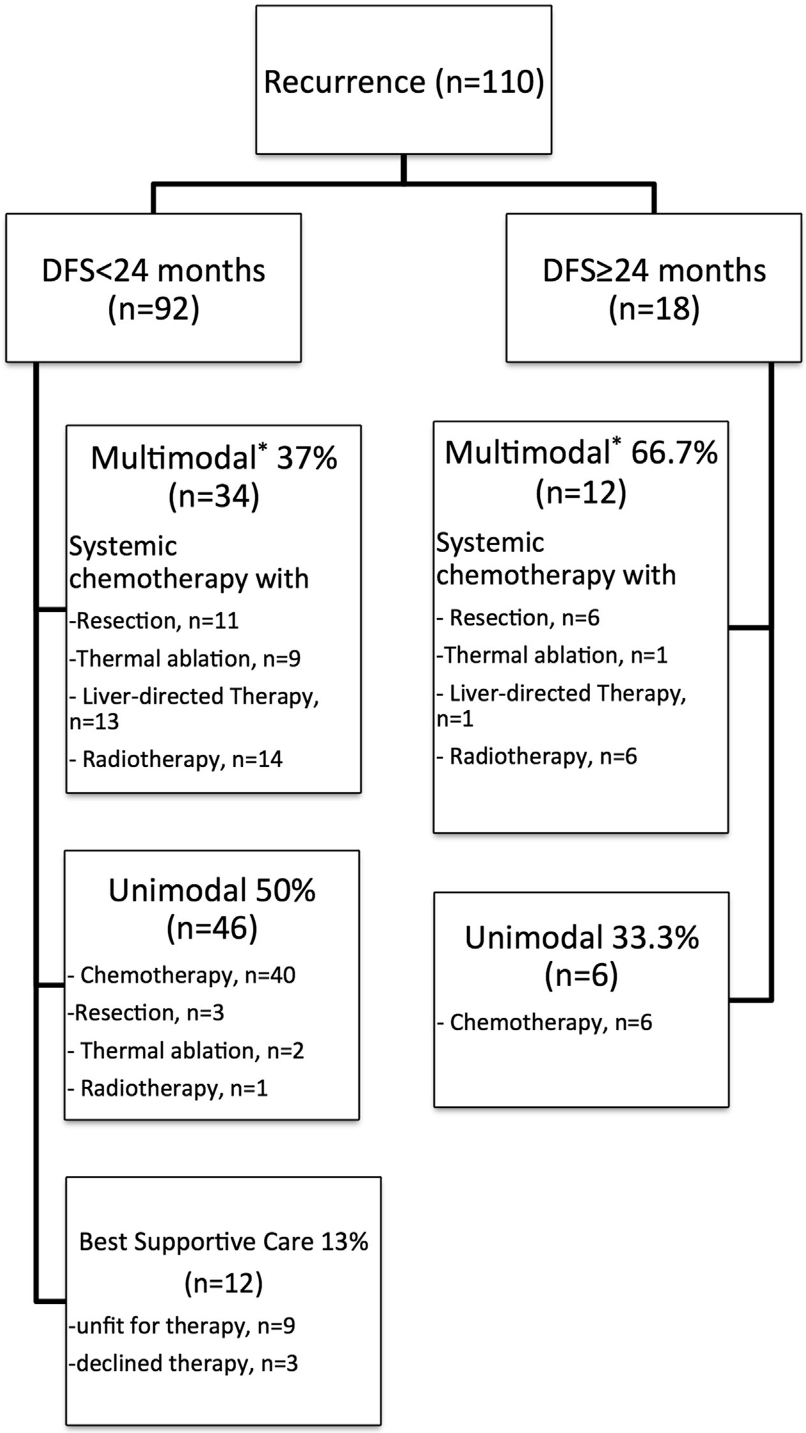


Figure 2. Recurrence management according to the recurrence patterns. *Patients might have undergone more than 2 different treatment modalities as multimodal therapy. DFS, disease-free survival.

online). When restricted to the subset of patients who underwent portal lymph node dissection (n = 92), the postoperative model performed similarly with significantly different median DFS across the different risk groups (low risk, 57.1 months; intermediate risk, 16 months; high risk, 8.2 months; $p < 0.001$, Fig. 5A).

Table 2. Univariable Analysis and Cox Proportional Hazards Regression Model of Preoperative and Postoperative Features Associated with Disease-Free Survival in the Primary Cohort (Memorial Sloan Kettering Cancer Center, n = 189 Patients)

	Univariable analysis				Multivariable analysis		
Variable	**Median disease-free survival, mo**	**HR**	**95% CI**	**p Value**	**HR**	**95% CI**	**p Value**
Preoperative							
Age	—	0.98	0.97–0.99	0.047	0.98	0.97–1.01	0.13
Sex							
Female	23.4	—	—	0.93	—	—	
Male	19.6	—	—		—	—	
Hepatitis							
Yes	16.6	—	—	0.11	—	—	
No	20	—	—		—	—	
PSC/IBD							
Yes	28.1	—	—	0.45	—	—	
No	17.8	—	—		—	—	
Preoperative tumor size	—	1.10	1.05–1.15	<0.001	1.09	1.04–1.14	<0.001*
Preoperative multiple tumor							0.013*
Yes	12	—	—	0.002	1.73	1.12–2.70	
No	23.4	—	—		—	—	
Preoperative enlarged lymph node							
Yes	16.9	—	—	0.66	—	—	
No	19.7	—	—		—	—	
Total bilirubin, mg/L	—	1.03	0.98–1.09	0.224			
Carcinogen antigen, 19-9, U/mL	—	1	1–1	0.004	1	1–1	0.3
Neoadjuvant therapy							
Yes	15.6	—	—	0.32	—	—	
No	20	—	—		—	—	
Postoperative							
Tumor size, cm	—	1.11	1.06–1.15	<0.001	1.10	1.05–1.15	<0.001*
Multiple lesions		—	—	<0.001	—	—	0.003*
Yes	13.2	—	—		1.82	1.22–2.71	
No	26.9	—	—		—	—	
Underlying liver							
Normal	16.9	—	—	0.66	—	—	
Steatosis	21	—	—		—	—	
Cirrhosis	23.7	—	—		—	—	
Tumor differentiation	—	—	—	0.037	—	—	0.79
Vascular invasion						—	
Absent	32	—	—	<0.001	Reference†	Reference†	0.022*
Micro	12.4	—	—		1.65	1.05–2.58	0.028*
Macro	9.6	—	—		1.93	1.13–3.31	0.016*
Perineural invasion							
Yes	15	—	—	0.008	1.26	0.80–1.98	0.32
No	20	—	—		—	—	
Extrahepatic invasion‡							
Yes	13.2	—	—	0.054	—	—	0.83
No	20.5	—	—		—	—	
Morphologic type							
Mass-forming	19.7	—	—	0.78	—	—	
Periductal invasion	17.8	—	—		—	—	

(Continued)

Table 2. Continued

	Univariable analysis				Multivariable analysis		
Variable	**Median disease-free survival, mo**	**HR**	**95% CI**	**p Value**	**HR**	**95% CI**	**p Value**
Margin status							
Negative	20	—	—	0.54	—	—	
Positive	19.5	—	—		—	—	
pN stage							
pN0	26.9	—	—	<0.001	Reference§	Reference§	<0.001*
pN1	8.2	—	—		2.77	1.52–5.03	<0.001*
pNx	20	—	—		1.03	0.69–1.53	0.89
Adjuvant therapy							
Yes	15	—	—	0.021	0.95	0.58–1.56	0.84
No	26.4	—	—		—	—	

All variables with $p > 0.1$ in univariable analysis were included in the Cox proportional hazards regression model.
*Significant.
†Patients with microvascular invasion and macrovascular invasion were respectively compared with patients without vascular invasion on tumor specimen.
‡Gallbladder excluded.
§pN1 and pNx patients were respectively compared with pN0 patients.
HR, hazard ratio; PSC/IBD, primary sclerosing cholangitis/inflammatory bowel disease.

External validation

The preoperative model allowed stratification in 2 risk groups significantly different in terms of median DFS (low risk, 26 months vs high risk, 13 months; $p < 0.001$, Fig. 3C). As compared with low-risk patients, patients in the high-risk group had a 117% greater likelihood of recurrence (HR = 2.17; 95% CI, 1.74–2.72; $p < 0.001$).

In turn, the postoperative model stratified the full cohort (n = 522) into 3 distinct risk groups in terms of median DFS (low risk, 48 months; intermediate risk, 18 months; high risk, 9 months; $p < 0.001$, Fig. 4D). As compared with low-risk patients, patients in the intermediate-risk group had a 90% greater likelihood of recurrence (HR = 1.9; 95% CI, 1.41–2.46; $p < 0.001$). In addition, patients classified into the high-risk group had a 199% greater likelihood of recurrence (HR = 2.99; 95% CI, 2.08–4.31; $p < 0.001$). When strictly applied to patients who underwent portal lymphadenectomy (n = 276), the postoperative model provided a similar stratification (median DFS in low-risk group 45 months; intermediate-risk group 18 months, and high-risk group 9 months; $p < 0.001$, Fig. 5B).

These distinct recurrence risk groups were also significantly different in terms of OS, as shown in the validation cohort (eFigure 1, available online).

DISCUSSION

The findings of the current study are important for a number of reasons. First, preoperative and postoperative prognostic models for patients with IHCC after curative-intent hepatectomy were developed and validated in a large external cohort. These models, easy to apply in clinical practice, allowed clear-cut classification of patients in groups of distinct outcomes both before and after resection. Second, distinct patterns of recurrences were identified. Recurrence within 24 months of resection overwhelmingly involved the liver (82.7%) and recurrences after 24 months were mostly isolated to an extrahepatic site.

Both preoperative and postoperative models allowed patients classification in groups with distinct recurrence rates and different DFS. Preoperative model was based on simple variables obtained on imaging. This model allowed classification in low-risk and high-risk groups (Figure 3). Patients deemed at high risk for recurrence had a 117% greater likelihood of recurrence with a significantly shorter median DFS (12 months) as compared with 31.1 months in the high-risk group ($p < 0.001$). In the validation cohort, preoperative model performed similarly. Postoperative model, including tumor features on pathology, stratified patients into 3 risk groups (low, intermediate, and high) and performed consistently in both the primary and validation cohorts (Fig. 4).

To date, 5 staging systems have been used successively for IHCC and several prognostic models and nomograms have been published recently and externally validated.[6,21,23,25,28-31] All are focused on OS estimation that remains the most relevant end point in clinical practice. Still, prognosis after resection of IHCC remains poor, mainly due to the high recurrence rate.[6,8,9] Hyder and colleagues[9] published a clinical risk score for recurrence,

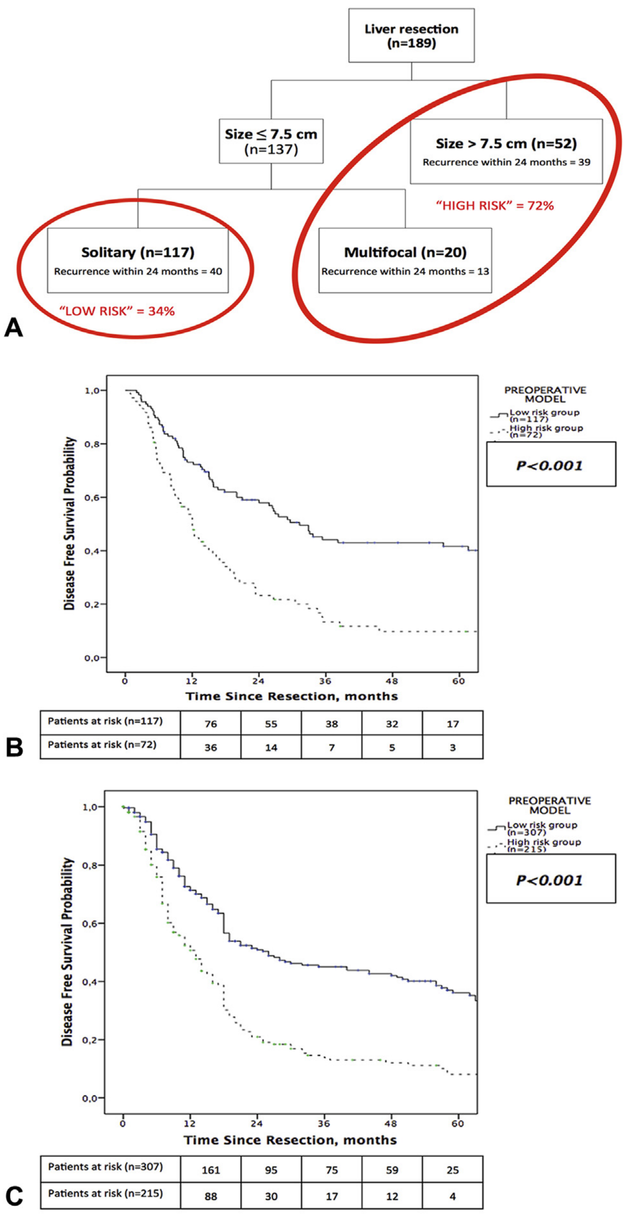
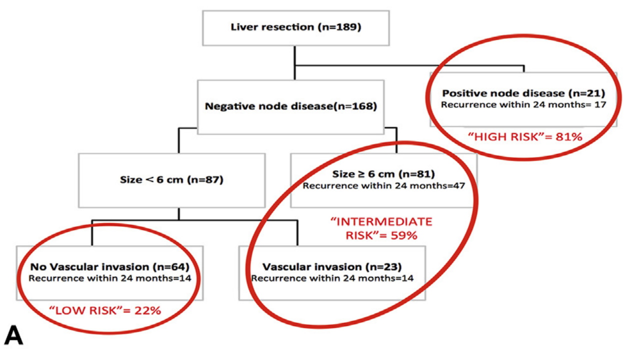


Figure 3. Preoperative model classifying patients into (A) recurrence risk groups and Kaplan-Meier estimates of disease-free survival for patients stratified by groups in the (B) primary cohort and (C) validation cohort.

including 3 items, such as tumor size $\geq$5 cm, major vascular invasion, and positive nodal disease.[9] They reported that an increasing risk score was associated with an incrementally worse DFS. However, this clinical score assigned equal strength (1 point) to each risk factor. In the current study, the risk of recurrence overtime varied as different independent prognostic factors were considered. For instance, based on our Cox regression analysis (Table 2), the probability of recurrence was 82% greater in case of multifocal disease on specimen ($p = 0.003$). This risk was 167% greater in case of positive nodal disease ($p < 0.001$). Using a recursive partitioning method, positive nodal disease was the most important variable in our postoperative model. Tumor size and vascular invasion helped to further classify patients without positive nodal disease. One can hypothesize that this method allowed respecting the different prognostic strength of each variable in our models.

Multifocal disease and tumor size, whether on imaging or pathology, were independent prognostic factors of shorter DFS. In the current study, tumor size estimation on preoperative imaging was found to be reliable, with a median difference between imaging and pathology (pathologic size − radiologic size) of + 0.41 cm. Regarding multifocality, according to Okabayashi and colleagues,[21] discrepancy between preoperative imaging and pathologic examination was observed in one-third of patients, but this discrepancy rate significantly decreased overtime. Of these 2 features, only multifocal disease is part of the current American Joint Committee on Cancer staging system.[23] In the postoperative model, tumor features, such as vascular invasion and positive nodal disease, replaced multifocal disease. Vascular invasion was previously reported as an independent predictor of recurrence.[9,24] As mentioned, positive nodal disease was the strongest independent predictor of short DFS. Its prognostic value has already been reported on extensively and routine portal lymphadenectomy is now widely recommended in recent guidelines.[3,32,33] In the primary cohort, nodal disease was suspected on the preoperative workup of 15 patients only (9.3%) and was not associated with DFS on univariable analysis ($p = 0.78$).

Resection remains the backbone of IHCC management, providing prolonged survival. Still, patients recurring after resection, such as those classified in the high-risk group, experienced median DFS ranging from 9 to 13 months (Figs. 3 and 4) and likely do not benefit from resection. Based on results from clinical trials in the palliative setting, current practice guidelines recommend adjuvant therapy in case of adverse tumor features (positive resection margin, presence of vascular invasion, positive nodal disease, multifocal disease). In the primary and validation cohorts, adjuvant chemotherapy was delivered to 43 patients (26.5%) and 178 patients (34.1%), respectively. Among them, 32 (62.7%) and 92 (51.7%) patients experienced recurrence within 24 months. In addition, adjuvant therapy was not independently associated with DFS. Taken altogether, these findings are not surprising, but they underscore that the main determinants of DFS are tumor characteristics and



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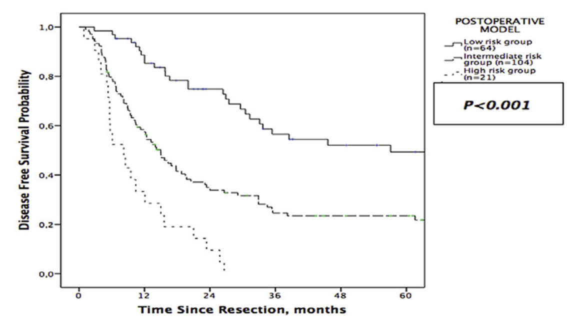
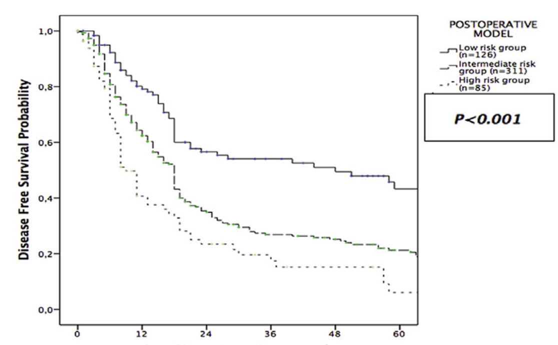


Patients at risk (n=64)	49	36	25	20	10
Patients at risk (n=104)	56	31	20	17	10
Patients at risk (n=21)	7	2	0	0	0

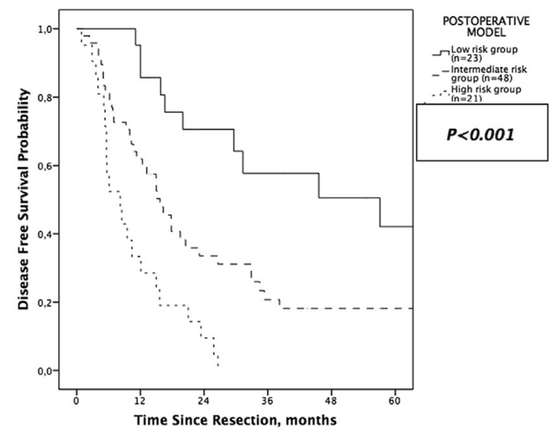
B



Patients at risk (n=126)	73	42	36	27	10
Patients at risk (n=311)	150	70	47	38	18
Patients at risk (n=85)	26	13	8	5	1

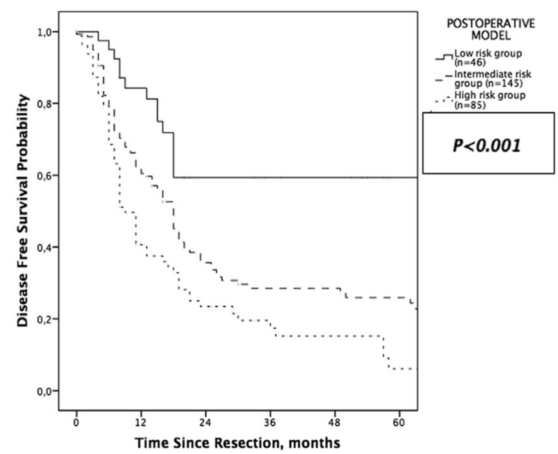
C

Figure 4. Postoperative model classifying patients into (A) recurrence risk groups and Kaplan-Meier estimates of disease-free survival for patients stratified by groups in the (B) primary cohort and (C) validation cohort.



Patients at risk (n=23)	18	11	8	6	2
Patients at risk (n=48)	27	13	7	4	2
Patients at risk (n=21)	7	2	0	0	0

A



Patients at risk (n=46)	26	15	13	10	5
Patients at risk (n=145)	71	34	23	20	11
Patients at risk (n=85)	26	13	8	5	1

B

Figure 5. Kaplan-Meier estimates of disease-free survival for patients who underwent portal lymphadenectomy classified using the postoperative model in (A) the primary cohort and (B) the validation cohort.

question the impact of adjuvant chemotherapy on recurrence. One clinical trial (ClinicalTrials.gov ID: NCT01313377) is currently investigating the impact of systemic therapy in the adjuvant setting.[34] However, given that recurrence often involves the liver, especially when occurring within 24 months after resection, targeted liver therapy might represent a credible option to increase disease control in the liver. Indeed, data from published clinical trials evaluating the impact of HAI-FUDR in unresectable IHCC reported a response rate of 48%, a hepatic progression-free survival reaching 12 months, and a median OS of 29 months.[11,12] Based on these compelling results, a phase II trial combining HAI-FUDR with systemic therapy (ClinicalTrials.gov ID: NCT01938729) in the adjuvant setting is currently accruing patients.[35] The validated preoperative and postoperative models can help in patient selection and inclusion in future clinical trials.

Although recurrence patterns are generally defined from anatomic sites, time to recurrence might represent a more relevant surrogate for tumor behavior. Most hepatic recurrence (91.5%) was seen in patients recurring within 24 months of resection. In contrast, most patients who were free of disease at 24 months had not recurred at time of last follow-up (73.5%), and recurrences were mainly observed at a solitary extrahepatic site (61.1%). This time dependence of recurrence patterns was also found in different patient subsets classified by recurrence risks. In other words, whatever the likelihood of recurrence for one patient, recurrence will be more likely to involve the liver or a distant organ when occurring within or after 24 months, respectively. In the primary cohort, recurrence management was generally more aggressive using a multimodal approach in patients who recurred after 24 months (n = 12 of 18 [66.7%]) than in those recurring earlier (n = 34 of 92 [33.3%]; p = 0.033). This finding might be due to the significantly different recurrence patterns between both groups. Indeed, recurrent disease within 24 months was simultaneously intrahepatic and extrahepatic (n = 26 of 92 [28.3%]), precluding multimodal management, while recurrences after 24 months were mostly isolated to a single organ (n = 17 of 18 [94.5%]), thereby allowing an aggressive approach with combined local and systemic therapies. The timing of recurrence might also have played some role in deciding the type of therapy, with a more aggressive approach favored in patients with a longer DFS. Similar to previous studies, a multimodal approach involving liver-directed therapies in selected patients was associated with prolonged survival in previous series.[36-39]

The current study had several limitations that should be addressed. First, the study is retrospective in nature, and reviewed data can be imprecise, especially for recurrence. Additionally, monitoring after IHCC resection is not standardized in France, even though a follow-up visit every 6 months for 5 years is generally advocated. This might represent a potential bias of differential recurrence screening. Second, predictive models that have been developed are easily applicable and all included prognostic variables are routinely available in clinical practice. One methodologic alternative would have been the development of a nomogram for DFS prediction. Third, portal lymph node dissection was performed in nearly half of patients. The association between nodal disease and recurrence could not be thoroughly explored in our study. However, the postoperative model performed similarly when strictly applied to patients who underwent portal lymphadenectomy in either the primary or the validation cohort. Finally, these models were developed from, and validated in, Western cohorts. As shown in Table 1, both cohorts were different for baseline characteristics, extent of resection, and tumor features. Such heterogeneity extends the applicability of these prediction tools to the daily clinical practice. However, additional validation might be needed before applicability on Eastern cohorts.

CONCLUSIONS

Recurrence patterns after resection for IHCC are time dependent. Preoperative and postoperative models as developed and validated in this study distinctly classified patients at different risk for recurrence. Patients classified as high risk might benefit from perioperative therapy instead of surgery alone.

Author Contributions

Study conception and design: Doussot, Gonen, Azoulay, Jarnagin
Acquisition of data: Doussot, Groot-Koerkamp, Wiggers
Analysis and interpretation of data: Doussot, Gonen, Farges, Azoulay, Jarnagin
Drafting of manuscript: Doussot, Gonen, Jarnagin
Critical revision: DeMatteo, Fuks, Allen, Farges, Kingham, Regimbeau, D'Angelica, Jarnagin

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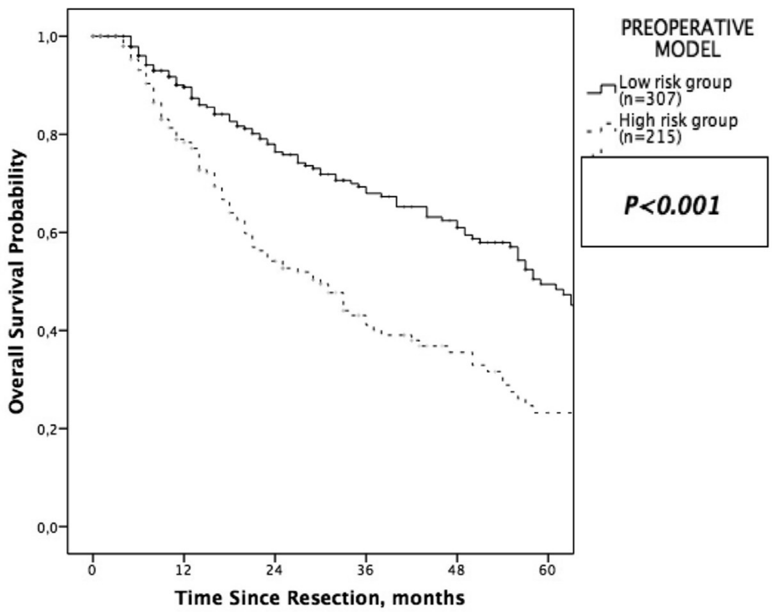
eTable 1. Recurrence Patterns According to Risk Groups in the Primary Cohort (Memorial Sloan Kettering Cancer Center, n = 189)

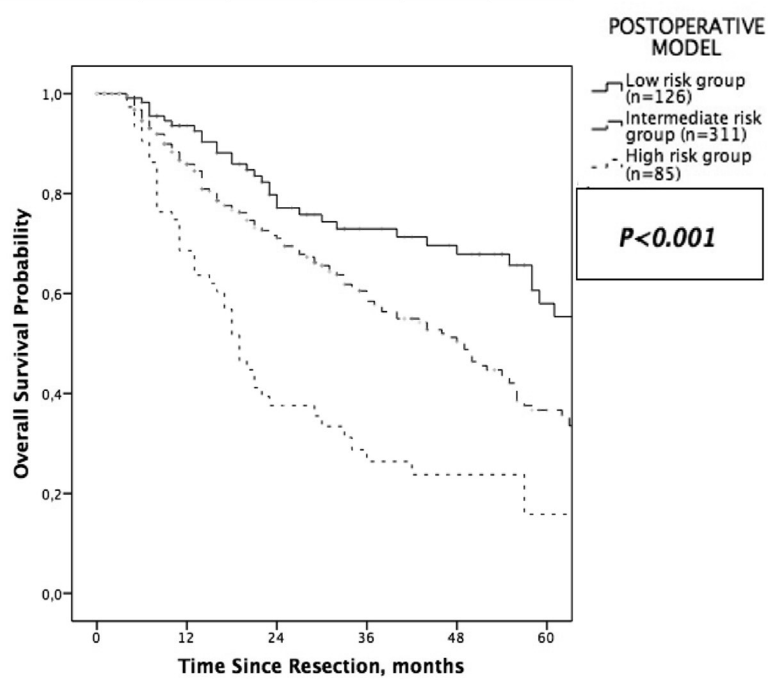
Variable	Overall recurrence rate (n = 110)		Within 24 mo recurrence rate (n = 92)		Hepatic recurrence within 24 mo, n/N† (n = 83)	After 24 mo recurrence rate (n = 18)		Extrahepatic recurrence after 24 mo, n/N‡ (n = 11)
	n*	%	n	%		n	%	
Preoperative model								
Low-risk group (n = 117)	54	46.2	40	74.1	34/40	14	25.9	9/14
High-risk group (n = 72)	56	77.8	52	92.8	42/52	4	7.1	2/4
Postoperative model								
Low-risk group (n = 64)	21	32.8	14	66.7	13/14	7	33.3	5/7
Intermediate-risk group (n = 104)	70	67.3	61	87.1	50/61	9	12.9	4/9
High-risk group (n = 21)	19	90.5	17	89.5	13/17	2	10.5	2/2

*n is the denominator for each risk group (each row).
†N is the denominator for each risk group (number of recurrence within 24 months).
‡N is the denominator for each risk group (number of recurrences after 24 months).



Patients at risk (n=307)	188	131	99	74	30
Patients at risk (n=215)	121	66	38	26	10

A



Patients at risk (n=126)	81	56	44	33	13
Patients at risk (n=311)	187	122	83	60	24
Patients at risk (n=85)	41	18	10	6	3

B

eFigure 1. Kaplan-Meier estimates of overall survival for patients from the validation cohort stratified using (A) the preoperative model and (B) the postoperative model.

Annexe 4.

Prognostic Genetic Alterations in Resected Intrahepatic Cholangiocarcinoma

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Prognostic Genetic Alterations in Resected Intrahepatic Cholangiocarcinoma

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Running head: Genetic Alterations in Resected ICC

SYNOPSIS

Sixty-six resected ICC specimens were assessed for genetic alterations using the Memorial Sloan Kettering-Integrated Mutation Profiling of Actionable Cancer Targets (MSK-IMPACT) sequencing assay. Gene alterations did not stratify risk of recurrence or death after resection for ICC; however, continued genomic profiling will facilitate identification of patients for molecular targeted therapies.

ABSTRACT

BACKGROUND: Resection is the only potentially curative treatment for intrahepatic cholangiocarcinoma (ICC). This study focused on characterizing the mutational landscape of resected ICC and identifying genetic markers that may be prognostic or suggestive of possible therapeutic intervention.

METHODS: Sixty-six resected ICC specimens were assessed for genetic alterations using the Memorial Sloan Kettering-Integrated Mutation Profiling of Actionable Cancer Targets (MSK-IMPACT) sequencing assay. Associations of gene mutations with survival outcomes were evaluated using the log-rank test and with histopathologic variables with logistic regression both adjusted for multiple comparisons with false discovery rate correction. Associations between histopathologic variables and outcomes were evaluated with univariable Cox regression.

RESULTS: The most common genetic alterations were *PBRM1* (24%), *IDH1* (23%), and *ARID1A* (21%). *KRAS* mutations (9%) and *FGFR2* fusions (8%) were relatively rare. Median OS was 53.4 months (95%CI:43.0-79.3) and median DFS was 17.4 months (95%CI:10.4-32.6). None of the gene alterations were associated with OS (p=0.29-0.84) or DFS (p=0.23-0.65); however, the chromatin remodeling gene family was associated with tumor size ≥5cm (OR:0.18,95%CI 0.05-0.65, p=0.044). Tumor size was prognostic of DFS only(OR:1.11,95%CI:1.03-1.20,p=0.009), while multifocal disease(OR:3.09,95%CI:1.66-5.75, OR:4.36,95%CI:2.05-9.28), positive lymph nodes(OR:5.40,95%CI:2.10-13.89, OR:8.10,95%CI:2.34-28.01), lymphovascular invasion(OR:1.93,95%CI:1.08-3.45, OR:2.23,95%CI:1.07-4.63), and periductal infiltration(OR:2.86,95%CI:1.31-6.26, OR:4.28,95%CI:1.77-10.39) were prognostic of both DFS and OS.

CONCLUSION: Gene alterations did not stratify risk of recurrence or death after resection for ICC. Histopathologic variables remain the most critical prognostic factors; however, continued genomic profiling will facilitate identification of patients for molecular targeted therapies, particularly for treatment of recurrent or metastatic disease.

INTRODUCTION

Intrahepatic cholangiocarcinoma (ICC) is an aggressive neoplasm arising from the epithelial lining of the intrahepatic biliary tract with increasing incidence and mortality worldwide[1]. In the United States, over the past three decades, the incidence of ICC has risen nearly four-fold, and has been accompanied by a parallel increase in mortality, reflecting the advanced stage at which ICC is usually diagnosed and the lack of effective treatments[2,3]. Resection is the only potential curative treatment and is associated with improved 5-year survival of up to 45%; however, the majority of patients present with unresectable disease[4-6].

Advances in gene sequencing have enabled exploration for patterns of genetic alterations that may hold both diagnostic and prognostic significance in ICC, as well as identify potentially actionable sites for molecular-targeted therapies[7-9]. However, given the rarity of ICC, occurring with an annual average incidence of 1/100,000 people, much of the current understanding has been derived from studies that have examined ICC within the context of other biliary tract cancers; these biliary tract cancers have subsequently been demonstrated to have significantly different genomic backgrounds and clinical behaviors. These previous studies have also encompassed a diverse study population, ranging from patients presenting with early, resectable disease to those presenting with metastatic disease[7,8,10]. These studies have identified significant inter-patient heterogeneity in the mutational profile of ICC, with mutations within individual genes occurring in less than one-third of the respective study populations. This significant genetic diversity poses a complication in furthering our understanding of the pathogenesis and prognosis of ICC and in the development of targeted therapeutic treatments.

This study focused specifically on characterizing the mutational landscape of patients with ICC undergoing resection. In using this select population, our goal was to reduce the potentially confounding effect of disease staging and to identify any potential prognostic genomic markers and clinicopathologic predictors that may be unique to patients with resectable disease.

METHODS

Patient Selection

This was a single-center study of patients with ICC who underwent resection with curative intent at Memorial Sloan Kettering Cancer Center (MSKCC) from January 1993 through December 2014. Patients were drawn from a larger study cohort, which only included patients with pre-operative contrast-enhanced computed tomography scans. Patients were selected for the present study if they either had previous genetic profiling by Memorial Sloan Kettering-Integrated Mutation Profiling of Actionable Cancer Targets (MSK-IMPACT) or had banked tumor available for MSK-IMPACT. The patient selection flowchart is shown in eFigure 1. This study was approved by the Institutional Review Board. The pre-, intra-, and post-operative management of these patients was conducted as detailed in our previous reports[6,11]. Patient data were collected from a prospectively-maintained database and supplemented with review of the electronic medical record. A pathologist (C.S.) masked to tumor genotype reviewed the archived hematoxylin and eosin stained slides of 66 patients to asses for periductal infiltration and steatosis. Steatosis was categorized as either present or absent, regardless of degree. Tumors were reassigned according to the American Joint Committee on Cancer 2010 TNM classification[12].

Tumor Sample Sequencing

Sequencing was performed using MSK-IMPACT, a customized array of 341 cancer-associated genes with subsequent expansion to 410 cancer-associated genes[13]. Fifteen of the 66 study patients had previous mutational profiling for clinical purposes and 51 patients had given informed consent for tissue to be used for genetic analysis. Of the 15 patients with previous MSK-IMPACT, 13 patients had sequencing performed using the 341-gene array, which was the largest panel available at the time, and two patients had sequencing using the 410-gene array. Slides were reviewed by a board-certified pathologist (E.V. or C.S.) to identify tumor and normal tissue for DNA extraction and sequencing. All samples had a minimum of 60% tumor content. Tumor and corresponding matched normal DNA were sequenced using the 410-gene array.

Statistical Analysis

Gene alterations occurring with greatest frequency were identified. Mutations were grouped by previously identified cancer pathways and families as follows: IDH1/2, chromatin-remodeling gene family (BAP1, ARID1A, PBRM1), DNA repair gene family (ATM, BRCA1, BRCA2, BAP1), RAS-MAPK pathway (RASA1, KRAS, NRAS, BRAF, MAPK1, MAPK3, MAP2K1, MAP3K1), TP53 and RAS-MAPK pathway, PI3K-AKT-mTOR pathway (PTEN, PIK3R2, PIK3CA, STK11, TSC1, RPTOR, MTOR, FBXW7), Notch signaling pathway (NOTCH4, FBXW7, EP300)[14-16]. Disease-free survival (DFS) was calculated from resection until recurrence or death. Patients alive and disease free at last follow-up were censored. Overall survival (OS) was calculated from the time of resection until death. Patients alive at last follow up were censored. Kaplan Meier plots and median and annual estimates with 95% log-log confidence intervals (CI) were provided.

Histopathologic variables were also evaluated for associations with DFS and OS using univariable Cox proportional hazards regression. The proportional hazards assumption was checked with martingale residuals. P-values were not adjusted for these known pathology/survival associations; unadjusted p-values<0.05 were considered significant.

Individual genes and genes within common pathways or families were evaluated for association with DFS and OS with the log-rank test. P-values for the gene groupings with each outcome were corrected using the false discovery rate approach (FDR). Adjusted p-values<0.05 were considered significant. Formal comparisons were done only for mutations in gene pathways and families occurring in at least 10 patients. Univariate logistic regression was used to assess the association between genetic pathways and pathologic outcomes. For the purpose of this analysis, tumor size was grouped into ≥5cm and <5cm. P-values were adjusted within each outcome.

As a post-hoc analysis, patients were dichotomized into early recurrence (<1year) versus late recurrence (≥1year). Two patients were censored prior to one year, and were considered in the late recurrence group for the purpose of this analysis. Fisher's exact test was used to assess the difference in genetic pathway presence based on early recurrence status, and p-values were adjusted with the FDR correction as well.

All tests were two-sided and all analyses were performed using SAS 9.4 (The SAS Institute, Cary, NC).

RESULTS

Patient Demographics and Treatment Characteristics

Sixty-six patients had tumor analyzed for genetic alterations. The majority of patients did not have a known history of viral hepatitis infection or pre-existing liver disease (41-92% negative). Margin-negative resection (R0) was achieved in 52 patients (78.8%). Lymphadenectomy was performed in 29 patients, of whom 12 patients (41.4%) had positive lymph nodes. Lymphovascular invasion was identified in 33 patients (50.0%), perineural invasion in 20 (30.3%) and periductal infiltration in eight (12.1%) (Table 1).

The majority of patients received additional therapies (n=47, 71.2%), including three patients who received neoadjuvant hepatic artery infusion pump therapy with 5-fluoro-2-deoxyuridine (HAI-FUDR), as part of a clinical trial protocol, and seven patients who received adjuvant HAI-FUDR. One patient also received subsequent treatment with yttrium-90 radioembolization. The majority of patients (61/66, 92%) had tumor samples obtained at the time of resection, prior to any treatment; five patients had tumor samples submitted for IMPACT obtained only after initiation of neoadjuvant therapies.

Patient Survival and Clinical Prognostic Factors

Median DFS was 17.4 months (95%CI:10.4-32.6months). Median OS was 53.4 months (95%CI:43.0-79.3months). 1-, 3-, and 5-year DFS were 55% (95%CI:79-95%), 29% (95%CI:57-80%), and 26% (95%CI:33-62%), respectively. 1-, 3-, and 5-year OS were 89% (95%CI:79-95%), 70% (95%CI:57-80%), and 48% (95%CI:33-62%), respectively.

Tumor size, multifocal disease, positive lymph nodes, lymphovascular invasion, and periductal infiltration were associated with DFS on univariable analyses (Table 2). Multifocal disease, positive lymph nodes, lymphovascular invasion, and periductal invasion were also associated with OS (Table 2).

Genetic Alterations

The median number of identified genetic alterations per patient sample was 3 (range 0-26). The most common genetic alterations were PBRM1 (24.2%), IDH1 (22.7%), and ARID1A (21.2%) (eTable 1). All 15 IDH1 mutations were missense mutations in the previously identified hotspot R132 and occurred mutually exclusively from IDH2 mutations[17-19]. Other common genetic alterations of interest included TP53 (12.1%), ATM (10.6%), BAP1 (10.6%), KRAS (9.1%), and RASA1 (9.1%). Five patients were also identified with FGFR2 gene fusions (7.6%). FGFR2 fusions occurred mutually exclusive from mutations in IDH1 and IDH2. A heat map displaying the occurrence of these gene alterations is shown in Figure 1.

The most commonly identified gene families and pathways include the chromatin-remodeling gene family (47.0%), the RAS-MAPK pathway (28.8%), IDH1/IDH2 (27.3%), and the mTOR pathway (21.2%).

Correlation between Mutational Status and Outcomes

None of the individual genetic alterations or gene families or pathways was significantly associated with either DFS or OS (Table 3). Figures 2 and 3 demonstrate DFS and OS stratified by alterations in IDH1/2, chromatin-remodeling genes, and FGFR2. The survival curves for patients with and without alterations in IDH1/2 overlap in both DFS and OS. For FGFR2 fusions, these were present in only five patients, limiting the ability to draw any significant comparisons in survival outcomes. Of note, all five patients had disease-free interval ≤2 years and presented with larger tumors, ranging 7-15 cm, including two with lymphovascular invasion and one with perineural invasion.

Patients with alterations in the chromatin-remodeling gene family trended towards improved DFS and OS compared with those who did not; however this was not statistically significant (Table 3). The presence of mutations in the chromatin-remodeling gene family was associated with lower odds of having a tumor size ≥5cm (OR:0.18, 95%CI:0.05-0.65,p=0 .044). There was no association between other genetic alterations and any tumor pathologic features (eTable 2).

In a post-hoc analysis, when patients were dichotomized into early compared with late recurrence subgroups, no differences were found based on the presence of genetic alterations (data not shown).

DISCUSSION

Unlike many other solid tumors that have a characteristic mutational profile, this study of resected ICC patients revealed no individual genes or gene groupings that were consistently mutated across the majority of tumors[20-23]. This is consistent with prior reports highlighting the genetic heterogeneity of ICC. However, certain gene groupings demonstrate a predilection for harboring alterations in ICC. The incidence of genetic alterations we identified in the chromatin-remodeling genes (47%), the RAS-MAPK pathway (28.8%), and in IDH1/2 (27.3%) was similar to previous reports[16,19,24,25]. However, the frequencies of individual gene alterations in KRAS (9.1%), TP53 (12.1%), and PBRMI (24.2%) in the present study are important to note when compared with previous reports. Prior studies have reported higher frequencies of KRAS (9-24%) and TP53 (3-38%) mutations and lower frequencies PBRM1 mutations (11%-17%)[8,10,16,25-27]. Differences in the frequency of these individual mutations are potentially a result of this study having a selected population of ICC patients submitted to curative resection. KRAS and TP53 have been highlighted as critical genes in the carcinogenic pathway and prognostic of poor clinical outcomes in other solid tumors including colorectal, pancreatic, and lung cancer [28-34]. The comparatively lower frequencies of KRAS and TP53 within the present study population may be a reflection of the typical association of the genes with more aggressive, and thus less likely to present as resectable, disease[8,35]. The higher frequency of PBRM1 mutations suggests potentially different carcinogenic pathways that may result in different clinical behaviors.

Further large-scale studies are needed to delineate the spectrum of phenotypes associated with these driver mutations within different subsets of ICC patients.

The present study is among the largest Western series reporting on the genetic alterations in ICC patients submitted to curative-intent resection. The next-generation sequencing assay used, MSK-IMPACT, is also unique in its extensive coverage of over 400 cancer-associated genes. In this resected population of ICC patients, we identified a significant association with the presence of mutations in chromatin-remodeling genes and a decreased likelihood of having tumor size ≥5cm. This subset of patients also trended towards having improved DFS and OS, reflecting the potential prognostic significance of the chromatin-remodeling genes that needs to be explored within larger resected ICC patient cohorts. Other studies evaluating the prognostic significance of mutations in chromatin-remodeling genes and the association with clinicopathologic characteristics have yielded variable results. Andrici et al identified an association between BAP1 mutations and increased tumor size, but also noted that BAP1 mutated tumors were less frequently associated with tumors presenting at an advanced stage and with lymphovascular invasion[36]. However, Jiao et al reported that patients with chromatin-remodeling gene mutations trended towards having decreased 3-year OS compared with those who did not (47.1% vs. 93.3%)[16]. These varying results may be due to differences in the presenting characteristics between these patient cohorts, again reflecting the intrinsic phenotypic and genomic heterogeneity of ICC. Collectively, these studies suggest a central role of the chromatin-remodeling genes in the carcinogenesis of ICC; the distinct roles of the individual genes and their effects on prognosis remain unclear.

Previous studies have produced conflicting results in the association between other commonly identified genetic alterations and patient outcomes. In examining the relationship between IDH1/2 mutations and DFS and OS, prior studies have suggested associations with improved survival, decreased survival, and also no association at all[8,16,18]. Kipp et al. reported an association between IDH mutations and poorly differentiated tumor histology; however, a subsequent study found no such association[24,37]. KRAS mutations have also been reported in prior studies to be associated with decreased survival, as well as perineural invasion[10,38]. However, these reports featured ICC populations with a much higher incidence of KRAS mutations (>20%) compared with the present study (9.1%). The present study did identify a significant proportion of ICC patients with mutations in the mTOR pathway (21.2%). The prominence of mutations within the mTOR pathway remains clinically significant as preliminary studies have demonstrated the potential therapeutic efficacy of mTOR pathway inhibition[39,40].

While none of the identified genetic alterations correlated with patient outcomes, pathologic features of tumor size, multifocal disease, lymph node status, lymphovascular invasion, and periductal infiltration, continued to demonstrate prognostic significance, consistent with previous studies[4,11,41]. One potential explanation is that when a patient undergoes resection, the phenotypic and pathologic features of the tumor become the more important outcome predictors. However, continued genomic profiling will facilitate personalized treatments with molecular-targeted therapies and enhance clinical trial participation. Ongoing investigation of inhibitor drugs targeting IDH mutations, FGFR2 fusions, and the mTOR pathway have demonstrated promising results for disease control in ICC patients and additional actionable targets continue to be explored[39,42-45]. These molecular-targeted therapies represent the crucial next step in the development of effective therapies for ICC both after resection and in the event of disease recurrence.

Limitations of this study include the inherent selection bias associated with a retrospective review and analysis of patients from a single institution, so these findings may not be generalized to all institutions. The low frequency of alterations within individual genes or gene families and pathways also limited our survival and subgroup analysis, including analysis by different adjuvant chemotherapy and locoregional treatments. Certain pathologic tumor characteristics were also unavailable for some specimens but this occurred in less than 10% of study patients.

CONCLUSION

The mutational landscape of ICC is highly heterogenous, even among early-stage, resectable patients. While genetic alterations alone did not stratify clinical outcomes, the comprehensive mapping of genomic alterations continues to hold significance for the development of molecular-targeted therapies and potentially for the treatment of recurrent disease. The pathologic features of the tumor remain the most crucial prognostic determinants, regardless of the underlying genomic profile.

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FIGURE LEGENDS

Figure 1. Heat map of genetic alterations of interest.

Figure 2. Correlation of genetic alterations of interest with disease free survival {a) IDH1/2, b) chromatin-remodeling gene family, c) FGFR2 fusions} and overall survival {d) IDH1/2, e) chromatin-remodeling gene family, f) FGFR2 fusions}.

eFigure 1. Study flow chart.

Table 1. Patient demographics and treatment characteristics

		All Patients N (%)
Demographics		
Age, years, median (range)		64.5 (28.7-86.9)
Gender	Male	25 (37.9)
	Female	41 (62.1)
Hepatitis-B Infection[a]	Yes	3 (4.5)
	No	35 (53)
	Unknown	28 (42.4)
Hepatitis-C Infection[b]	Yes	3 (4.5)
	No	27 (40.9)
	Unknown	36 (54.5)
Liver Cirrhosis	Present	5 (7.6)
	Absent	61 (92.4)
Liver Steatosis	Present	24 (36.4)
	Absent	42 (63.6)
Operative Details and Pathologic Features		
Resection Procedure	Left Hepatectomy	19 (28.8)
	Right Hepatectomy	8 (12.1)
	Extended Left Hepatectomy	6 (9.1)
	Extended Right Hepatectomy	14 (21.2)
	Segmentectomy	19 (28.8)
Tumor Size, median (range)		6.0 (2.2-24.0)
T-Stage	1	22 (33.3)
	2A	18 (27.3)
	2B	11 (16.7)
	3	7 (10.6)
	4	6 (9.1)
	Unknown	2 (3)
Tumor Grade	Well-Differentiated	1 (1.5)
	Moderately-Differentiated	46 (69.7)
	Poorly-Differentiated	18 (27.3)
	Unknown	1 (1.5)
Multifocal Disease	Present	16 (24.2)
	Absent	50 (75.8)
Margin Status	R1	14 (21.2)
	R0	52 (78.8)
Lymph Node Status	Positive	12 (18.2)
	Negative	17 (25.8)
	Unknown	37 (56.1)

		All Patients N (%)
Lymphovascular Invasion	Present	33 (50)
	Absent	33 (50)
Perineural Invasion	Present	20 (30.3)
	Absent	44 (66.7)
	Unknown	2 (3)
Periductal Infiltration	Present	8 (12.1)
	Absent	57 (86.4)
	Unknown	1 (1.5)
Liver Capsule Involvement	Present	6 (9.1)
	Absent	58 (87.9)
	Unknown	2 (3)
Adjacent Organ Involvement	Present	2 (3)
	Absent	63 (95.5)
	Unknown	1 (1.5)
Additional Treatment		
Any Adjuvant Therapy	Yes	47 (71.2)
	No	19 (28.8)
HAI-FUDR	Yes	10 (15.2)
	No	56 (84.8)
Y-90	Yes	1 (1.5)
	No	65 (98.5)

HAI-FUDR indicates hepatic artery infusion 5-fluoro-2-deoxyuridine; Y=Yttrium.

[a]Viral hepatitis B infection was defined by presence of Hepatitis B surface antigen in the patient's serum.

[b]Viral hepatitis C infection was defined by presence of HCV antibodies in the patient's serum.

Table 2. Univariable analysis of pathologic features with DFS/OS.

Pathologic Feature		DFS #T(#E)	DFS HR	DFS [95%	CI]	DFS p-value	OS #T(#E)	OS HR	OS [95%	CI]	OS p-value
Tumor Size		65(50)	1.11	[1.03-	1.20]	**0.009**	65(33)	1.09	[0.98-	1.21]	0.10
Tumor Size	≥5cm	48(38)	1.42	[0.74-	2.73]	0.30	48(26)	1.35	[0.58-	3.15]	0.48
	<5cm	17(12)	REF				17(7)	REF			
Multifocal Disease	Present	16(15)	3.09	[1.66-	5.75]	**<.001**	16(12)	4.36	[2.05-	9.28]	**<.001**
	Absent	50(36)	REF				50(21)	REF			
Tumor Grade	Poor	18(13)	0.88	[0.47-	1.65]	0.68	18(10)	1.15	[0.54-	2.45]	0.71
	Well/Moderate	47(37)	REF				47(23)	REF			
Margin Status	R1	14(11)	0.90	[0.45-	1.82]	0.77	14(8)	1.05	[0.44-	2.48]	0.91
	R0	52(40)	REF				52(25)	REF			
Lymph Note Status	Positive	12(12)	5.40	[2.10-	13.89]	**<.001**	12(9)	8.10	[2.34-	28.01]	**<.001**
	Negative	17(12)	REF				17(5)	REF			
Lymphovascular Invasion	Positive	33(28)	1.93	[1.08-	3.45]	**0.026**	33(18)	2.23	[1.07-	4.63]	**0.031**
	Negative	33(23)	REF				33(15)	REF			
Perineural Invasion	Positive	20(18)	1.65	[0.91-	3.00]	0.10	20(10)	1.76	[0.81-	3.84]	0.15
	Negative	44(31)	REF				44(22)	REF			
Periductal Infiltration	Positive	8(8)	2.86	[1.31-	6.26]	**0.008**	8(7)	4.28	[1.77-	10.39]	**0.001**
	Negative	57(43)	REF				57(26)	REF			

#T=total number of patients within level; #E=number of patients with an event within level; HR=hazard ratio

Table 3. Kaplan-Meier analysis for gene family/pathway with DF/OS.

		DFS					OS				
Gene Family/ Pathway	**Mutation Status**	**#T(#E)**	**5 Yr. Est**	**[95%**	**CI]**	**p-value***	**#T(#E)**	**5 Yr. Est**	**[95%**	**CI]**	**p-value***
IDH1	Present	15(10)	0.32	[0.09-	0.58]	0.26	15(6)	0.65	[0.35-	0.84]	0.31
	Absent	51(41)	0.24	[0.13-	0.38]		51(27)	0.44	[0.28-	0.60]	
IDH1+IDH2	Present	18(12)	0.37	[0.14-	0.61]	0.23	18(7)	0.71	[0.44-	0.87]	0.31
	Absent	48(39)	0.21	[0.10-	0.35]		48(26)	0.41	[0.25-	0.57]	
ARID1A	Present		NR				14(3)	0.59	[0.08-	0.90]	0.31
	Absent	52(44)	0.24	[0.13-	0.37]		52(30)	0.45	[0.30-	0.59]	
PBRM1	Present	16(12)	0.24	[0.06-	0.48]	0.65	16(9)	0.50	[0.22-	0.73]	0.84
	Absent	50(39)	0.27	[0.15-	0.41]		50(24)	0.49	[0.31-	0.64]	
Chromatin-Remodeling Gene Family	Present	31(20)	0.32	[0.15-	0.51]	0.13	31(12)	0.60	[0.35-	0.77]	0.09
	Absent	35(31)	0.20	[0.09-	0.36]		35(21)	0.38	[0.20-	0.56]	
ARID1A+PBRM1	Present	27(17)	0.31	[0.13-	0.50]	0.23	27(10)	0.55	[0.27-	0.75]	0.31
	Absent	39(34)	0.24	[0.11-	0.38]		39(23)	0.43	[0.26-	0.60]	
RAS-MAPK Pathway	Present	19(16)	0.21	[0.06-	0.42]	0.23	19(11)	0.32	[0.10-	0.56]	0.31
	Absent	47(35)	0.28	[0.16-	0.43]		47(22)	0.56	[0.38-	0.71]	
RAS-MAPK+TP53 Pathway	Present	24(21)	0.21	[0.07-	0.40]	0.23	24(16)	0.29	[0.11-	0.50]	0.29
	Absent	42(30)	0.29	[0.15-	0.45]		42(17)	0.61	[0.42-	0.76]	
PI3K-AKT-mTOR Pathway	Present	14(13)	0.21	[0.05-	0.45]	0.23	14(9)	0.27	[0.07-	0.53]	0.31
	Absent	52(38)	0.28	[0.15-	0.41]		52(24)	0.55	[0.38-	0.70]	
DNA Repair Gene Family	Present	14(11)	0.29	[0.07-	0.55]	0.65	14(6)	0.66	[0.32-	0.86]	0.33
	Absent	52(40)	0.26	[0.14-	0.39]		52(27)	0.43	[0.27-	0.59]	
BAP1*	Present	7(5)	0.34	[0.05-	0.69]		7(3)	0.83	[0.27-	0.97]	
	Absent	59(46)	0.26	[0.15-	0.38]		59(30)	0.44	[0.28-	0.58]	
KRAS*	Present		NR					NR			
	Absent	60(45)	0.29	[0.18-	0.42]		60(29)	0.53	[0.37-	0.66]	
RASA1*	Present	6(3)	0.63	[0.14-	0.89]		6(2)	0.56	[0.07-	0.88]	
	Absent	60(48)	0.24	[0.13-	0.36]		60(31)	0.48	[0.32-	0.62]	

Gene Family/ Pathway	Mutation Status	DFS #T(#E)	DFS 5 Yr. Est	DFS [95%	DFS CI]	DFS p-value*	OS #T(#E)	OS 5 Yr. Est	OS [95%	OS CI]	OS p-value*
TP53*	Present	8(8)	0.13	[0.01-	0.42]		8(8)	0.13	[0.01-	0.42]	
	Absent	58(43)	0.29	[0.17-	0.42]		58(25)	0.56	[0.39-	0.69]	
Notch Signaling Pathway*	Present	6(5)	0.33	[0.05-	0.68]		6(4)	0.50	[0.11-	0.80]	
	Absent	60(46)	0.26	[0.15-	0.38]		60(29)	0.49	[0.33-	0.63]	
FGFR2 Fusions*	Present		NR				5(4)	0.27	[0.01-	0.69]	
	Absent	61(46)	0.29	[0.17-	0.41]		61(29)	0.50	[0.35-	0.64]	

#T=total number of patients within level; #E=number of patients with an event within level; CI=confidence interval; 5-Yr. Est=5-year Kaplan Meier estimate; NR=estimate not reached.

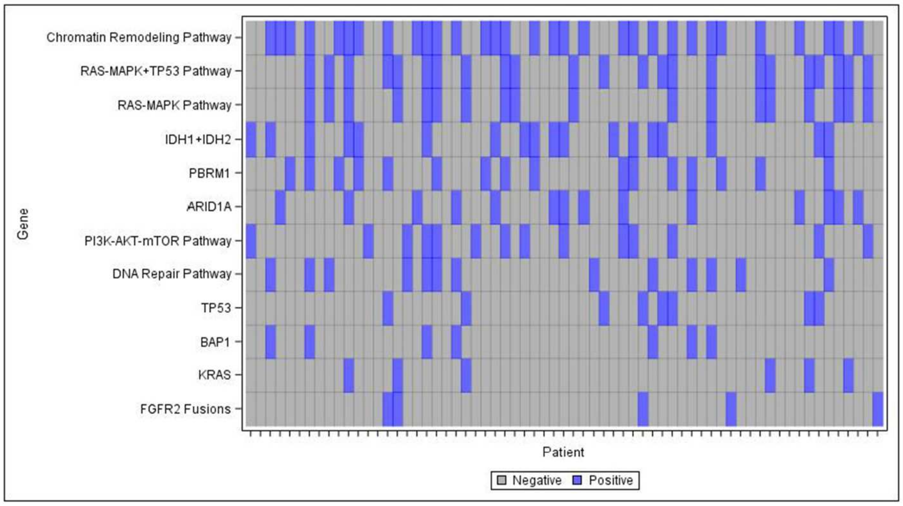


Figure 1. Heat map of genetic alterations of interest.

279x215mm (88 x 88 DPI)

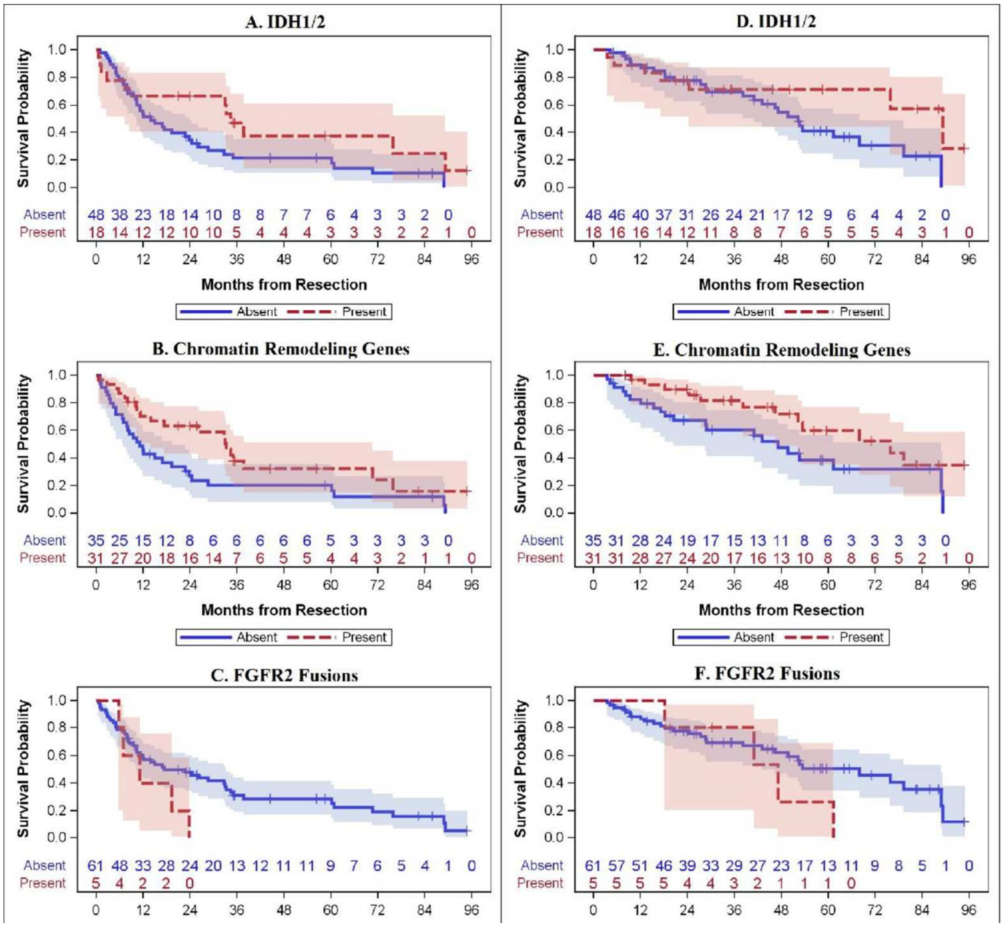


Figure 2. Correlation of genetic alterations of interest with disease free survival {a) IDH1/2, b) chromatin-remodeling gene family, c) FGFR2 fusions} and overall survival {d) IDH1/2, e) chromatin-remodeling gene family, f) FGFR2 fusions}.

530x486mm (60 x 60 DPI)

Supplemental Table 1. Frequency of mutations by individual genes.

	N (%)
PBRM1	16 (24.2)
IDH1	15 (22.7)
ARID1A	14 (21.2)
TP53	8 (12.1)
ATM	7 (10.6)
BAP1	7 (10.6)
KRAS	6 (9.1)
RASA1	6 (9.1)
FGFR2	5 (7.6)
FBXW7	3 (4.5)
IDH2	3 (4.5)
NOTCH4	3 (4.5)
PTEN	3 (4.5)
BRAF	2 (3)
MAPK3	2 (3)
NRAS	2 (3)
PIK3CA	2 (3)
PIK3R2	2 (3)
RPTOR	2 (3)
EP300	1 (1.5)
MAP2K1	1 (1.5)
MAP3K13	1 (1.5)
MTOR	1 (1.5)
STK11	1 (1.5)
TSC1	1 (1.5)

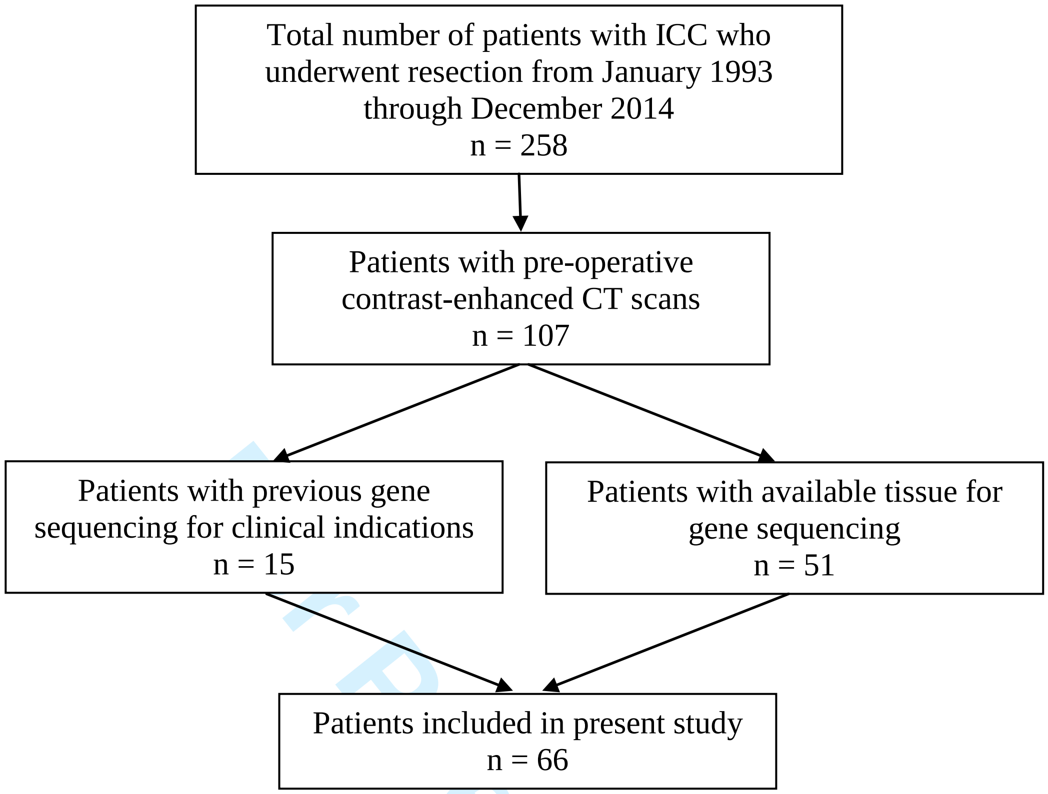
Supplemental Table 2. Univariable logistic regression for gene mutations with pathologic features.

Pathologic Feature	Pathway		Total(#Pos)	OR	[95% CI]	p-value*
Tumor Size (grouped)	IDH1	Present	15 (11)	0.97	[0.26 - 3.57]	>0.95
		Absent	50 (37)	REF		
	IDH1+IDH2	Present	18 (11)	0.42	[0.13 - 1.38]	0.38
		Absent	47 (37)	REF		
	ARID1A	Present	14 (7)	0.24	[0.07 - 0.86]	0.09
		Absent	51 (41)	REF		
	PBRM1	Present	16 (10)	0.48	[0.14 - 1.63]	0.48
		Absent	49 (38)	REF		
	Chromatin Remodeling Gene Family	Present	31 (18)	0.18	[0.05 - 0.65]	**0.044**
		Absent	34 (30)	REF		
	ARID1A+PBRM1	Present	27 (15)	0.19	[0.06 - 0.63]	**0.044**
		Absent	38 (33)	REF		
	RAS-MAPK Pathway	Present	19 (14)	0.99	[0.29 - 3.33]	>0.95
		Absent	46 (34)	REF		
	RAS-MAPK+TP53 Pathway	Present	24 (18)	1.1	[0.35 - 3.49]	>0.95
		Absent	41 (30)	REF		
	PI3K-AKT-mTOR Pathway	Present	14 (10)	0.86	[0.23 - 3.20]	>0.95
		Absent	51 (38)	REF		
	DNA Repair Gene Family	Present	14 (11)	1.39	[0.34 - 5.72]	>0.95
		Absent	51 (37)	REF		
Satellite Nodules	IDH1	Present	15 (4)	1.18	[0.32 - 4.40]	0.94
		Absent	51 (12)	REF		
	IDH1+IDH2	Present	18 (4)	0.86	[0.24 - 3.11]	0.94
		Absent	48 (12)	REF		
	ARID1A	Present	14 (2)	0.45	[0.09 - 2.28]	0.94
		Absent	52 (14)	REF		
	PBRM1	Present	16 (4)	1.06	[0.29 - 3.89]	0.94
		Absent	50 (12)	REF		
	Chromatin Remodeling Gene Family	Present	31 (5)	0.42	[0.13 - 1.38]	0.94
		Absent	35 (11)	REF		
	ARID1A+PBRM1	Present	27 (5)	0.58	[0.18 - 1.91]	0.94
		Absent	39 (11)	REF		
	RAS-MAPK Pathway	Present	19 (4)	0.78	[0.22 - 2.81]	0.94

		Absent	47 (12)	REF		
	RAS-MAPK+TP53 Pathway	Present	24 (6)	1.07	[0.33 - 3.42]	0.94
		Absent	42 (10)	REF		
	PI3K-AKT-mTOR Pathway	Present	14 (3)	0.82	[0.20 - 3.39]	0.94
		Absent	52 (13)	REF		
	DNA Repair Gene Family	Present	14 (3)	0.82	[0.20 - 3.39]	0.94
		Absent	52 (13)	REF		
Margins	IDH1	Present	15 (5)	2.33	[0.64 - 8.50]	0.66
		Absent	51 (9)	REF		
	IDH1+IDH2	Present	18 (6)	2.5	[0.72 - 8.64]	0.66
		Absent	48 (8)	REF		
	ARID1A	Present	14 (3)	1.02	[0.24 - 4.29]	>0.95
		Absent	52 (11)	REF		
	PBRM1	Present	16 (1)	0.19	[0.02 - 1.58]	0.66
		Absent	50 (13)	REF		
	Chromatin Remodeling Gene Family	Present	31 (6)	0.81	[0.25 - 2.66]	0.91
		Absent	35 (8)	REF		
	ARID1A+PBRM1	Present	27 (4)	0.50	[0.14 - 1.82]	0.74
		Absent	39 (10)	REF		
	RAS-MAPK Pathway	Present	19 (4)	0.99	[0.27 - 3.64]	>0.95
		Absent	47 (10)	REF		
	RAS-MAPK+TP53 Pathway	Present	24 (6)	1.42	[0.43 - 4.72]	0.81
		Absent	42 (8)	REF		
	PI3K-AKT-mTOR Pathway	Present	14 (4)	1.68	[0.44 - 6.47]	0.75
		Absent	52 (10)	REF		
	DNA Repair Gene Family	Present	14 (4)	1.68	[0.44 - 6.47]	0.75
		Absent	52 (10)	REF		
Lymphadenopathy	IDH1	Present	6 (3)	1.56	[0.26 - 9.47]	>0.95
		Absent	23 (9)	REF		
	IDH1+IDH2	Present	7 (3)	1.08	[0.19 - 6.06]	>0.95
		Absent	22 (9)	REF		
	ARID1A	Present	5 (1)	0.30	[0.03 - 3.05]	0.92
		Absent	24 (11)	REF		
	PBRM1	Present	7 (3)	1.08	[0.19 - 6.06]	>0.95
		Absent	22 (9)	REF		
	Chromatin Remodeling Gene Family	Present	13 (4)	0.44	[0.10 - 2.06]	0.92

		Absent	16 (8)	REF		
	ARID1A+PBRM1	Present	12 (4)	0.56	[0.12 - 2.60]	0.92
		Absent	17 (8)	REF		
	RAS-MAPK Pathway	Present	9 (4)	1.2	[0.24 - 5.89]	>0.95
		Absent	20 (8)	REF		
	RAS-MAPK+TP53 Pathway	Present	12 (5)	1.02	[0.23 - 4.57]	>0.95
		Absent	17 (7)	REF		
	PI3K-AKT-mTOR Pathway	Present	5 (3)	2.5	[0.35 - 17.94]	0.92
		Absent	24 (9)	REF		
	DNA Repair Gene Family	Present	3 (2)	3.2	[0.26 - 40.06]	0.92
		Absent	26 (10)	REF		
Lymphovascular Invasion	IDH1	Present	15 (6)	0.59	[0.18 - 1.91]	0.87
		Absent	51 (27)	REF		
	IDH1+IDH2	Present	18 (7)	0.54	[0.18 - 1.63]	0.87
		Absent	48 (26)	REF		
	ARID1A	Present	14 (6)	0.69	[0.21 - 2.28]	0.87
		Absent	52 (27)	REF		
	PBRM1	Present	16 (8)	1	[0.32 - 3.08]	>0.95
		Absent	50 (25)	REF		
	Chromatin Remodeling Gene Family	Present	31 (12)	0.42	[0.16 - 1.13]	0.87
		Absent	35 (21)	REF		
	ARID1A+PBRM1	Present	27 (12)	0.69	[0.26 - 1.84]	0.87
		Absent	39 (21)	REF		
	RAS-MAPK Pathway	Present	19 (10)	1.16	[0.40 - 3.37]	>0.95
		Absent	47 (23)	REF		
	RAS-MAPK+TP53 Pathway	Present	24 (13)	1.30	[0.48 - 3.55]	0.87
		Absent	42 (20)	REF		
	PI3K-AKT-mTOR Pathway	Present	14 (8)	1.44	[0.44 - 4.73]	0.87
		Absent	52 (25)	REF		
	DNA Repair Gene Family	Present	14 (7)	1.00	[0.31 - 3.26]	>0.95
		Absent	52 (26)	REF		
Perineural Invasion	IDH1	Present	14 (2)	0.30	[0.06 - 1.47]	0.56
		Absent	50 (18)	REF		
	IDH1+IDH2	Present	17 (3)	0.38	[0.09 - 1.51]	0.56
		Absent	47 (17)	REF		
	ARID1A	Present	13 (4)	0.97	[0.26 - 3.63]	>0.95

	Absent	51 (16)	REF		
PBRM1	Present	16 (5)	1	[0.30 - 3.39]	>0.95
	Absent	48 (15)	REF		
Chromatin Remodeling Gene Family	Present	30 (9)	0.9	[0.31 - 2.59]	>0.95
	Absent	34 (11)	REF		
ARID1A+PBRM1	Present	26 (9)	1.3	[0.45 - 3.79]	>0.95
	Absent	38 (11)	REF		
RAS-MAPK Pathway	Present	17 (8)	2.59	[0.82 - 8.24]	0.56
	Absent	47 (12)	REF		
RAS-MAPK+TP53 Pathway	Present	22 (9)	1.95	[0.65 - 5.82]	0.58
	Absent	42 (11)	REF		
PI3K-AKT-mTOR Pathway	Present	14 (5)	1.3	[0.37 - 4.52]	>0.95
	Absent	50 (15)	REF		
DNA Repair Gene Family	Present	14 (4)	0.85	[0.23 - 3.13]	>0.95
	Absent	50 (16)	REF		


Total number of patients with ICC who underwent resection from January 1993 through December 2014
n = 258
Patients with pre-operative contrast-enhanced CT scans
n = 107
Patients with previous gene sequencing for clinical indications
n = 15
Patients with available tissue for gene sequencing
n = 51
Patients included in present study
n = 66

PLOS | ONE

RESEARCH ARTICLE

Circulating Plasma Levels of MicroRNA-21 and MicroRNA-221 Are Potential Diagnostic Markers for Primary Intrahepatic Cholangiocarcinoma

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Abstract

Background

MicroRNAs (miRNAs) are potential biomarkers in various malignancies. We aim to characterize miRNA expression in intrahepatic cholangiocarcinoma (ICC) and identify circulating plasma miRNAs with potential diagnostic and prognostic utility.

Methods

Using deep-sequencing techniques, miRNA expression between tumor samples and non-neoplastic liver parenchyma were compared. Overexpressed miRNAs were measured in plasma from an independent cohort of patients with cholangiocarcinoma using RT-qPCR and compared with that healthy volunteers. The discriminatory ability of the evaluated plasma miRNAs between patients and controls was evaluated with receiving operating characteristic (ROC) curves.

Results

Small RNAs from 12 ICC and 11 tumor-free liver samples were evaluated. Unsupervised hierarchical clustering using the miRNA expression data showed clear grouping of ICC vs. non-neoplastic liver parenchyma. We identified 134 down-regulated and 128 upregulated miRNAs. Based on overexpression and high fold-change, miR21, miR200b, miR221, and miR34c were measured in plasma from an independent cohort of patients with ICC (n = 25) and healthy controls (n = 7). Significant overexpression of miR-21 and miR-221 was found

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in plasma from ICC patients. Furthermore, circulating miR-21 demonstrated a high discriminatory ability between patients with ICC and healthy controls (AUC: 0.94).

Conclusion

Among the differentially expressed miRNAs in ICC, miR-21 and miR-221 are overexpressed and detectable in the circulation. Plasma expression levels of these miRNAs, particularly miR-21, accurately differentiates patients with ICC from healthy controls and could potentially serve as adjuncts in diagnosis. Prospective validation and comparison with other hepatobiliary malignancies is required to establish their potential role as diagnostic and prognostic biomarkers.

Introduction

Intrahepatic cholangiocarcinoma (ICC) is the second most common primary hepatic malignancy after hepatocellular carcinoma but its age-adjusted incidence is constantly rising [1,2]. The only potentially curative treatment is complete resection, which offers a median overall survival approximating 30 months [3–6]. Due to a nonspecific presentation, most patients are diagnosed at an advanced stage precluding resection. Moreover, clinical and radiologic differentiation from other primary liver tumors (both malignant and benign) and from metastatic disease can be challenging and the value of percutaneous diagnostic biopsy is uncertain [7]. Identifying non-invasive and reliable biomarkers that aid in early diagnosis would represent a significant advance, especially in high-risk populations. The ideal diagnostic biomarker is readily measurable, minimally invasive, reproducible, and highly accurate at identifying the disease at hand. There are no reliable diagnostic biomarkers for ICC currently endorsed in clinical practice, and the only established prognostic factors are pathologic features requiring invasive tumor tissue procurement.

MicroRNAs (miRNAs) are small non-coding RNAs that modulate the gene expression at the post-transcriptional level in a sequence-specific manner. Functional studies have shown miRNAs to participate in almost every cellular process including apoptosis, proliferation and differentiation by directly modulating the expression of tumor suppressor genes and oncogenes [8–10]. A potential diagnostic, prognostic and therapeutic value of miRNA expression profiles in ICC has been reported [11–13]. Most recently, Zhang et al reported a microarray study where they identified a 30-miRNA signature which distinguished ICC from normal biliary epithelium, and a 3-miRNA signature that accurately predicted prognosis in patients diagnosed with ICC with an area under the curve (AUC) of 0.747. [14]

Certain miRNAs are stable and easily measurable in serum and plasma and therefore hold the potential to be ideal cancer biomarkers [15–17]. Promising results have been seen using circulating miRNAs as predictors of outcome in various malignancies [18–22]. However, there is a paucity of studies evaluating circulating miRNAs specifically as markers of ICC.

The goal of the current study was to find circulating markers of ICC. To this end, we used historical FFPE (formalin fixed paraffin embedded) tumor samples and deep sequencing techniques to determine the miRNA expression profile of ICC in two independent cohorts of patients (exploratory and validation). Furthermore, aiming to identify circulating miRNAs that are abnormally elevated in the setting of ICC, deregulated miRNAs were measured in plasma from an independent group of patients with ICC to establish feasibility as potential circulating diagnostic markers of the disease.

Methods

Patient samples

Authorization was obtained from our institutional review board (IRB) and the human biospecimen utilization committee (HBUC). The hepatopancreatobiliary surgery database was queried to identify patients with ICC, and this query was crossmatched with the Department of Pathology's tissue procurement services (TPS) database to identify availability of tissue. Patients with available tumor tissue, tumor-free liver parenchyma, and in whom preoperative plasma had been collected were identified. All patients had provided informed consent for tissue banking. Tumor tissue and non-tumoral hepatic parenchyma were collected during exploratory laparotomy for liver resection in patients with resectable disease, or hepatic arterial infusion (HAI) pump placement for those with unresectable disease at presentation. Most patients with unresectable ICC were treated with HAI of floxuridine, with or without intravenous bevacizumab as part of two phase II clinical trials previously published [23,24]. For studies on circulating miRNA, plasma samples were obtained from a control cohort of healthy volunteers over the age of 40 with no diagnosis of cancer. Ten milliliters of whole blood from patients and controls were collected in ethylenediaminetetraacetic acid (EDTA) coated tubes and spun down within 30 minutes of collection to retrieve the plasma. Blood collection was performed within seven days before resection in resected patients or before treatment initiation in unresected patients. Plasma was stored in 1ml aliquots and preserved at -80 degrees Celsius until analysis. Solid tissue obtained from TPS were collected directly from the operating room or pathology suite and stored either as formalin fixed paraffin embedded (FFPE) blocks, or frozen at -80 degrees Celsius according to established protocols and keeping strict track of processing times [25]. To ensure adequate RNA integrity, we selected specimens with less than 2 hours from collection to freezing.

RNA extraction

Whole-section of frozen tumor samples were pulverized in liquid nitrogen and homogenized in Trizol solution followed by RNA isolation according to the manufacturer's instructions (Life technologies). Whole-section curls were obtained from FFPE tissue blocks without microdissection and processed after deparaffinization steps using the RecoverAll kit (Life technologies). RNA was extracted from plasma samples following the mirVana platform as previously published [26,27]. Three steps of phenol/chloroform purification were added to increase purity of the RNA samples.

Small RNA sequencing

Small RNA libraries were generated using the TruSeq kit from Illumina and subjected to deep sequencing using the Hi-Seq 2000 Illumina platform. The reads were then mapped to the human genome (hg19 build), and the normalized expression of each miRNA was determined using the DESeq software [28].

Expression levels of individual miRNAs were compared between cholangiocarcinoma and companion non-tumor bearing liver samples. miRNAs with log expression change p-value < 0.0001 by paired t-test, at a false-discovery rate (FDR) of 1%, were considered differentially expressed.

Isolation and quantification of circulating miRNAs

For normalization purposes, plasma samples from healthy controls and patients with ICC were spiked with synthetic RNA oligos corresponding to C. elegans miR-39, miR-54, and miR-213 at a final concentration of 10 fmol/ml prior to RNA extraction. Expression levels of the selected human miRNAs were determined by RT-qPCR using specific Taqman primers (Applied Biosystems) and normalized to expression of the "spiked-in" C. elegans miRNAs, as previously described [26]. The discriminatory ability of the evaluated plasma miRNAs between cholangiocarcinoma patients and healthy volunteers was evaluated with receiving operating characteristic (ROC) and area under the curve (AUC) analysis.

Statistical analysis

Differences of miRNA expression between groups were calculated by the t-test or the Mann-Whitney U test as appropriate. All tests were two-tailed. A value of $P < 0.05$ was considered to indicate a statistically significant difference. Concordance index was calculated using receiver operating characteristic (ROC) curves. All analyses were performed using Stata/IC 12.0 (StataCorp LP, College Station, TX). Graphs were created using the GraphPad Prism 6.0 software (San Diego, CA, USA).

Results

Comparison of small RNA libraries obtained from FFPE and frozen specimens

To determine whether small RNA sequencing from FFPE archival samples could provide reliable results, we first generated and sequenced small RNA libraries from five ICC patients in whom both frozen and FFPE samples were available thus allowing a paired comparison. The FFPE blocks RNA yield ranged between 46 and 52 ng/ml with RNA integrity numbers (RIN) between 4.3 and 7.2. Fig 1 shows two representative pairs of samples showing adequate correlation between FFPE and frozen tissue (Spearman's pairwise correlation: 0.66, 0.91, 0.78, 0.75, 0.43; all $P < 0.0001$- t test), indicating that high-quality small RNA sequencing data can be obtained from FFPE.

Sequencing and identification of candidate biomarkers

We extracted and sequenced small RNAs from 12 additional FFPE ICC and 11 tumor-free liver samples. On average we obtained 9.5×10^6 reads mapped to known miRNAs per sample (min = 8.2×10^5 reads; max = 18.7×10^6 reads). Unsupervised hierarchical clustering using the miRNA expression data showed clear grouping of ICC specimens vs. normal liver (Fig 2).

The analysis of these data revealed 262 microRNAs that were differentially expressed in tumor compared to tumor-free liver parenchyma (at a false discovery rate of 1%). Of these, 134 were down regulated and 128 were upregulated (Fig 3). To increase the likelihood of identifying useful plasma biomarkers, we focused on miRNAs with the highest degree of upregulation in the tumor samples (fold-change in comparison to tumor-free liver) and that were among the most abundant in terms of absolute expression levels. Based on these criteria, we selected 4 miRNAs (miR-21, miR-34c, miR-200b, and miR-221) for further analysis in plasma (Fig 4).

Tissue validation of overexpression and exploration of circulating miRNAs

An independent set of FFPE tumors (10 samples) and tumor-free liver parenchyma (5 samples) was used as a validation cohort. Real-time quantitative polymerase chain reaction (RT-qPCR) was used to confirm the differential expression of our candidate miRNAs (miR-21, miR-34c, miR-200b, and miR-221) Fig 5A. MiR-21, miR-34c, and miR-200b were also overexpressed in this cohort. Interestingly, miR-34c absolutely discriminated tumor samples from non tumor-

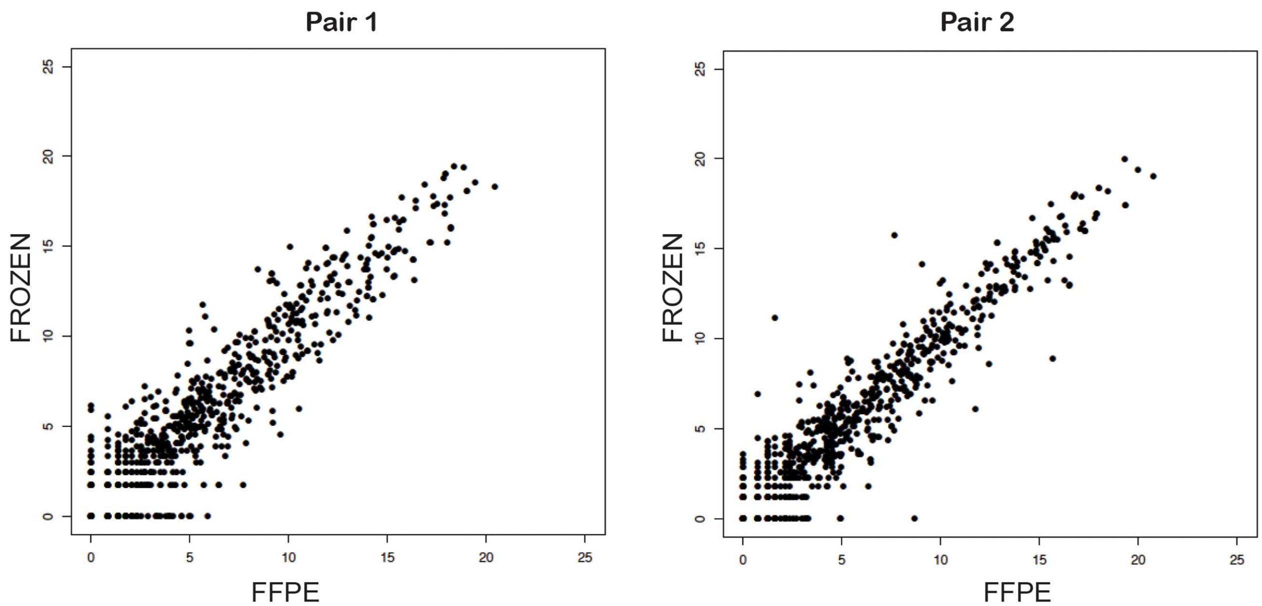

Fig 1. Representative scatter plots of normalized miRNA expression showing the correlation between FFPE and frozen paired tumor samples.

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bearing liver. While miR-221 was found in larger concentration in the ICC samples, this difference did not reach statistical significance.

The potential role of these up-regulated candidate miRNAs as circulating biomarkers of ICC was assessed using RT-qPCR in plasma samples of 25 patients with ICC of different grades and plasma from 7 healthy controls. The normalized C_T (threshold cycle) values for the evaluated miRNAs in plasma from cases and controls are shown in Table 1. There was no statistical significance, regarding miR-34c and miR-200b, which found in very low quantity in the plasma of ICC patients whereas undetectable in controls. Overexpression of miR-21 and miR-221 in plasma from an independent cohort of patients with ICC is shown in Fig 5B. Discrimination between healthy controls and ICC patients, was excellent using circulating miR-21 expression (AUC = 0.94; Fig 6).

Clinicopathological correlation and prognostic value of circulating miRNAs

A total of 35 patients with clinicopathological and follow up data and were analyzed for prognostic evaluation. Descriptive data are listed in Table 2. Of these, 24 patients underwent a liver resection; they had not reached a median overall survival after a median follow-up of 28 months (IQR 10–63). Fifteen patients (65%) experienced recurrence. Circulating miR-221 expression was significantly higher in poorly differentiated tumors (n = 5) than in moderately differentiated tumors (n = 19; p = 0.016). No other tumor features such as tumor size, multiple lesions, lymphovascular invasion, nodal status, resection status correlated with miR-21 and 221 expression profiles. The remaining 11 patients had irresectable disease and were treated with hepatic arterial infusion of Floxuridine. Partial tumor response was seen in 55% and their median overall survival was 17 months (IQR 14–32). There was no association between miRNA expression levels and tumor size, multiple lesions, extrahepatic disease, or response to HAI chemotherapy. Neither of these groups displayed correlation between survival outcomes and miRNA expression levels.

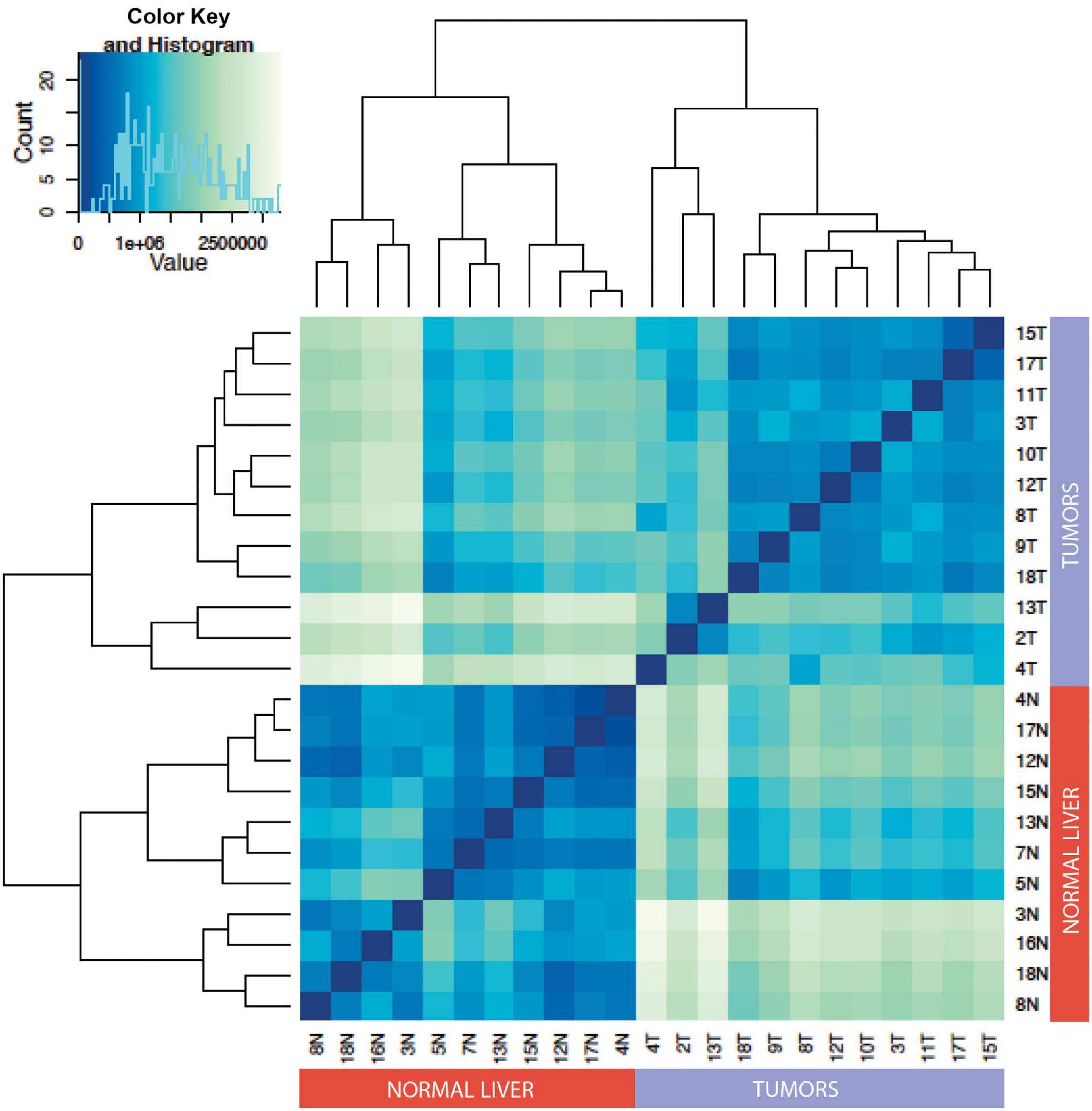

Fig 2. Correlation matrix heatmap showing the Euclidean distance between non-tumor bearing liver and ICC samples. Darker color indicates stronger correlation.

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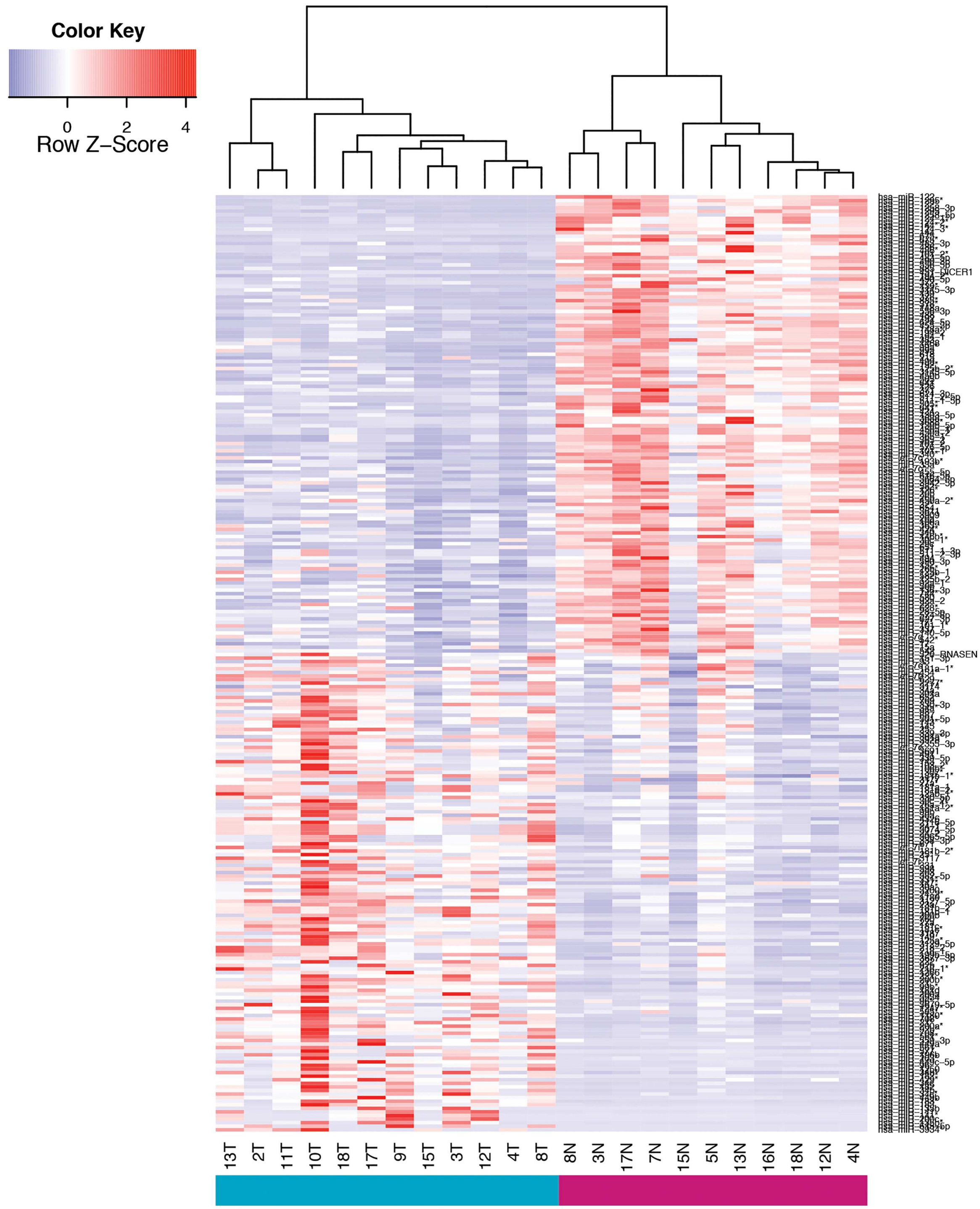

Fig 3. Heat map of the differentially expressed miRNAs (adjusted p value <0.001) between ICC and non-tumor bearing liver FFPE samples.

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Discussion

Using high-throughput small RNA sequencing, we have defined the spectrum of miRNAs expressed in ICC and compared them to those in normal liver. We have identified a subset of

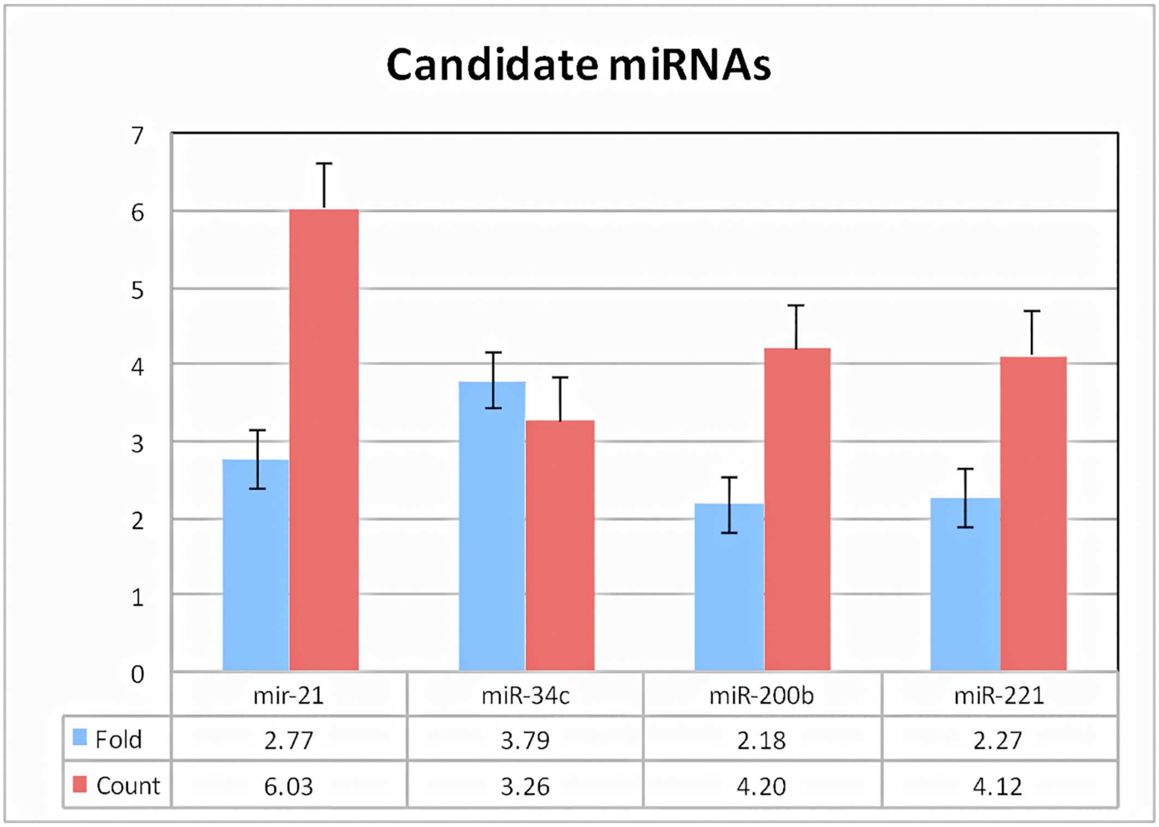

Fig 4. Selected miRNAs based on the highest degree of up regulation (fold change, blue columns) and absolute expression counts (red columns). Fold change was calculated between ICC and non tumor-bearing liver. Error bars represent standard error of the mean.

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differentially expressed miRNAs—namely, miR-21, miR-34c, miR-200b, and miR-221. Furthermore, we have established that miR-21 and miR-221 are highly upregulated in ICC tumor tissue and can be detected in plasma from patients with ICC at higher concentrations compared to healthy individuals, thus suggesting their potential as diagnostic markers.

MiR-21 is known as a commonly deregulated microRNA in a variety of malignancies including lung, gastric, esophageal, colorectal, breast, prostate, pancreas, renal, glioblastoma, hepatocellular carcinoma, among others [12,21,29–40]. MiR-21 expression levels in tissue have been shown to adequately segregate cholangiocarcinoma tissue samples from normal tissues [12,41,42]. Furthermore, miR-21 has been shown to be a potential diagnostic circulating biomarker in different solid malignancies. [43] However, to our knowledge there are no previous reports showing that this miRNA is of diagnostic value in the plasma from patients with ICC.

In our patient population, among the 4 miRNAs that were selected as diagnostic candidates, miR-21 showed the highest absolute expression, while still maintaining a nearly 3-fold expression change in comparison to tumor-free liver parenchyma. This difference was maintained in the plasma samples with a striking and statistically significant difference between patients and healthy controls. When plotted in a receiver operating characteristic (ROC) curve to asses its ability as a diagnostic marker, miR-21 displayed an elevated concordance index (AUC: 0.94) which underscores its potential role as a diagnostic marker for patients with intrahepatic cholangiocarcinoma. Our findings are in line with those recently reported by Wang et al [44], in

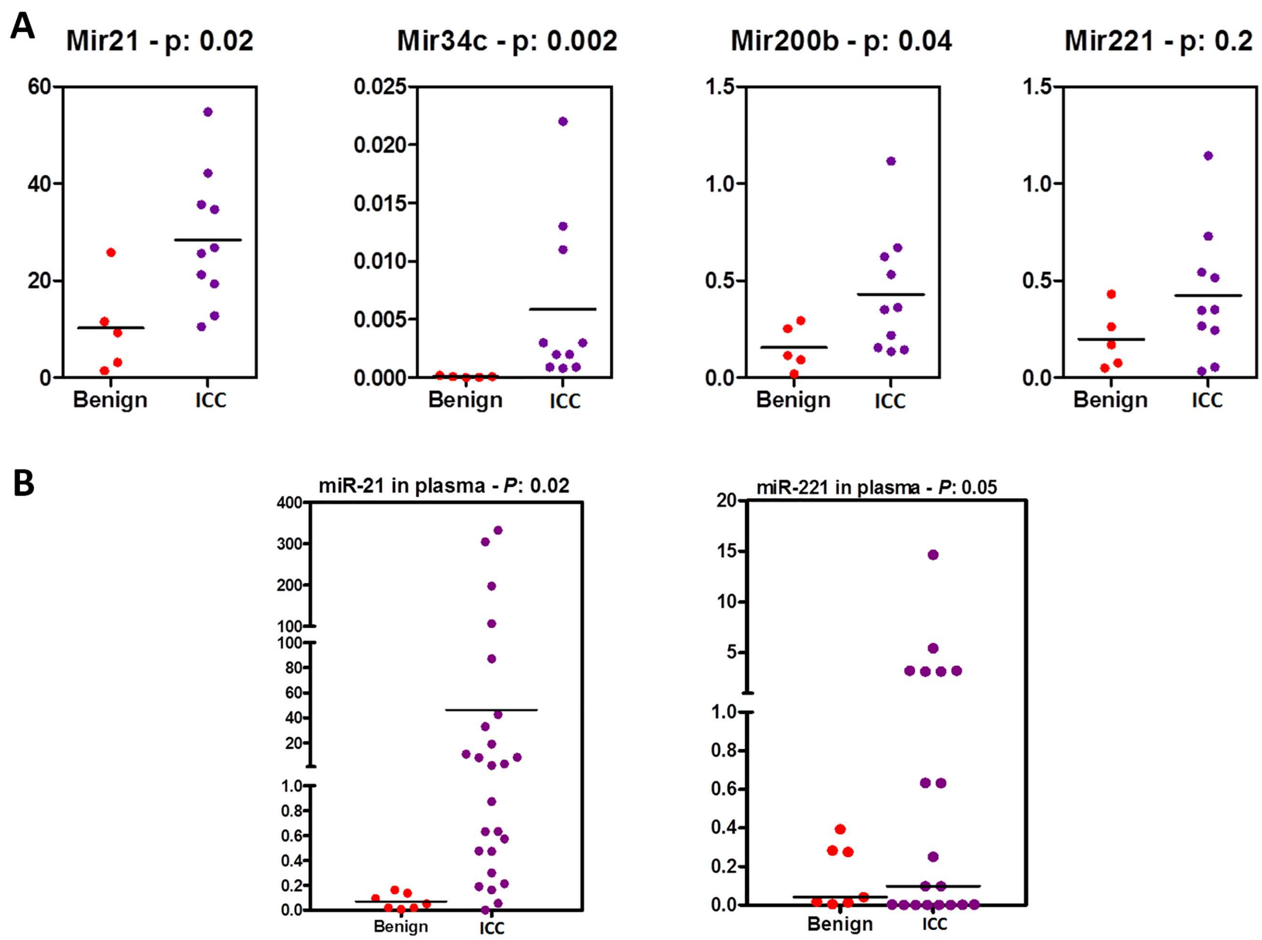

Fig 5. RT-qPCR validation of differentially expressed miRNAs in tissue (A) and plasma (B). (A) differential expression of miR-21, miR-34c, miR-200b and miR-221 on tissues from 10 independent patients with ICC and 10 normal liver samples. Horizontal bars represent mean value. (B) Expression levels of circulating miR-21 and miR-221 in an independent cohort of patients with ICC vs healthy controls.

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their experience, serum miR-21 segregated patients with ICC from healthy controls with an AUC: 0.94.

MiR-21 is established as an oncogenic microRNA (oncomiR) [45]. Potential mechanisms include PTEN (phosphatase and tensin homolog) tumor suppressor gene downregulation

Table 1. Normalized expression of selected miRNAs in plasma.

microRNA	Patients		Controls		P value
	Mean	SD	Mean	SD	
miR-34c	0.019	0.05	Undetectable		NA
miR-200b	0.158	0.405	Undetectable		0.4
miR-21	43.881	93.421	0.070	0.062	0.0001
miR-221	1.657	3.584	0.147	0.164	0.05

Comparison of normalized expression of selected miRNAs in plasma of patients with ICC and healthy controls. Expression was measured with RT-qPCR and normalized with spiked-in C. elegans miR-39, miR-54, and miR-213.

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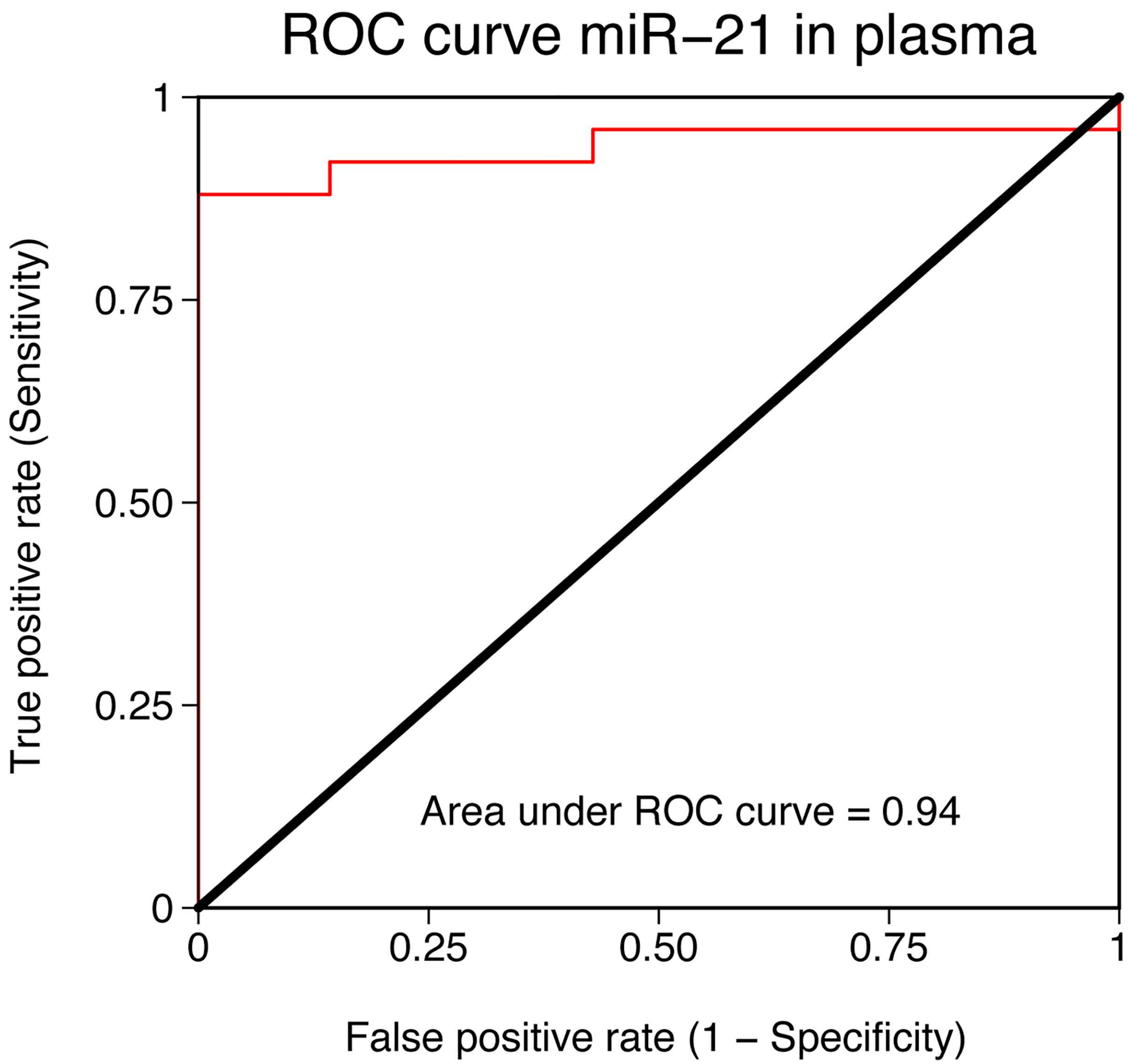

Fig 6. Receiver operating characteristic (ROC) curve for circulating miR-21 for the diagnosis intrahepatic cholangiocarcinoma. ROC was performed for plasma miR-21 from patients with ICC vs healthy controls. Area under the curve (AUC) for miR-21 is 0.94.

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[46,47], decreasing the Bax/Bcl-2 ratio and caspase-3 activity thus negatively modulating apoptosis [48], as well as translational repression of the tumor suppressor PDCD4 (programmed cell death 4) [42,49] and downregulation of TIMP3 (tissue inhibitor of metalloproteinases 3) which is thought to function as an inhibitor of metastasis [42]. In the study by Wang et al, mechanistic roles for miR-21 were identified whereby its inhibition resulted in suppression of ICC cell proliferation in vitro and in vivo by induction of cell cycle arrest and apoptosis. They also identified PTEN, in addition to PTPN14 as functional targets of miR-21. [44] Moreover, miR-21 overexpression has been associated with increased invasiveness and ability to metastasize in cholangiocarcinoma cell lines [50]. While the latter suggests a possible prognostic role for miR-21 in ICC, there is currently no prospective data on large clinical samples to support this hypothesis. In the current study we identified no correlation between oncologic outcomes

Table 2. Clinicopathological features of ICC patients.

	Resected (n = 24)	Unresected (n = 11)	Whole cohort (n = 35)
Age, years	64 (13)	56 (12)	62 (13)
Female (%)	10 (46%)	5 (46%)	16 (46%)
Total Bilirubin, mg/l	1.4 (3.2)	0.9 (0.6)	1.2 (2.5)
Albumin, g/l	4.1 (0.4)	4.1 (0.3)	4.1 (0.4)
Median CA 19–9, U/l (IQR)	18.5 (61)	151 (7918)	37.5 (144)
Median CEA, U/l (IQR)	2.3 (1.8)	10 (132)	2.2 (2.6)
Multiple lesions (%)	2 (9%)	5 (45%)	9 (26%)
Largest Tumor Size, cm	5.6 (2.7)	8.9 (4.4)	6.6 (3.5)
Tumor Differentiation			
Poor	5 (21%)		
Moderate	19 (79%)		
Lymphovascular Invasion (%)			-
Micro	11 (45.8%)		
Macro	11 (45.8%)		
Nodal Status (%)			
pNx	14 (58.3%)		
pN0	8 (33.3%)		
pN1	2 (8.3%)		
Positive Margin Status (%)	23 (95.8%)		
Adjuvant Therapy (%)	6 (25%)		
Recurrence (%)	15 (62.5%)		
Response to HAI FUDR (%)			
Progression		1 (9.1%)	
Stable		4 (36.4%)	
Partial response		6 (54.5%)	

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and the expression levels of these miRNAs, however, our samples size, and the heterogeneity of treatments over time limit the power of this observation.

Similarly, circulating miR-221 was differentially expressed between ICC and healthy patients. It has been reported that miR-221 is associated with a variety of malignancies including bladder, gastric, hepatocellular carcinoma, non-small cell lung cancer, but has not been reported as extensively as miR-21overexpression in ICC [18,51–53]. Potential roles of miR-221 have been reported in many cancers such as hepatocellular carcinoma formation in cirrhotic liver by targeting the tumor suppressor DNA-damage inducible transcript 4 (DDIT4), modulating the mTOR pathway, or in bladder cancer cells by modulating p53 upregulated modulator of apoptosis [54,55]. Interestingly, while miR-221 is generally considered an oncogenic miRNA, it has been recently reported as having tumor-suppressive effects in certain non small cell lung cancer cell lines by potentially inducing intra-S-phase arrest and/or apoptosis [56].

Given the oncogenic or tumor-suppressor activity of various microRNAs in different malignancies [9,57,58], several experimental approaches to profile miRNA expression in tissue samples and in biological fluids have been reported [26,27,59]. The most popular, due to the relative low cost and the limited technical complexity, are microarray based approaches and quantitative-RT-PCR methods. These methods however have important limitations. First, the analysis is limited to small RNAs for which specific probes are available, thus preventing the discovery of novel miRNAs. In addition, the cross hybridization between closely related miRNAs, often limits the specificity of the assays. Finally, these techniques do not allow the identification of base-substitution or deletion/insertion in the small RNAs, thus severely limiting the opportunity to identify unknown mutations with oncogenic potential.

In the current study, we avoided these limitations by performing an unbiased assessment of the microRNA profile of paired clinical samples of ICC and normal liver tissue using direct next-generation sequencing. Because the relative abundance of a miRNA is directly proportional to the number of sequence reads mapping, and because miRNAs differing at a single base position can be easily distinguished using this approach, the results of these experiments provide a highly detailed map of the miRNAs present in the samples analyzed.

One of the advantages of miRNAs as potential biomarkers is their high stability in body fluids, enabling their use as non invasive diagnostic and prognostic tools. Increased circulating levels of some of the microRNAs that we identified in our study have been found to be differentially expressed in patients affected by other tumor types, including breast (miR-21), esophageal (miR-21), gastric (miR-21) and lung cancer (miR-221), melanoma (miR-221), hepatocellular carcinoma (miR-21 and miR-221) [12,18,20–22,60]. Hence, extrapolating these miRNAs as exclusive diagnostic biomarkers of ICC in the general population might prove to be inaccurate, especially to differentiate ICC and hepatocellular tumors. Accordingly, further investigations are warranted for validation, notably for screening populations at risk of ICC with primary sclerosing cholangitis, primary biliary sclerosis, viral hepatitis, liver cirrhosis or parasitic biliary disease.

In clinical practice, it is often challenging to establish the nature of liver masses based on preoperative imaging and biopsies alone. Our results show that miR-21 and miR-221 detection in the plasma can discriminate between healthy individuals and patients affected by ICC. Our study has several limitations: Most notably, the ability of these markers to discriminate ICC from other malignant liver disease has not been shown. Also, given the small sample size used and the heterogeneity of treatments over the years, the assessment of the potential prognostic and/or predictive role for the identified microRNAs, or their correlation with established pathologic markers of disease severity and aggressiveness is limited. This would require a larger, prospective evaluation of patients with a range of diagnoses as biological controls.

Conclusion

In this exploratory study, we identified a set of deregulated microRNAs in ICC. Among them, miR-21, miR-34c, miR-200b, and miR-221 are overexpressed. Furthermore, miR-21 and miR-221 are detectable in the circulation and clearly overexpressed in patients with ICC compared with healthy controls. For miR-21, these plasma expression levels accurately differentiate patients with ICC from controls and could potentially serve as adjuncts in diagnosis. Further studies, will address the need for prospective validation and comparison with other hepatobiliary malignancies to confirm the findings presented here and assess their potential role as prognostic biomarkers.

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Author Contributions

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Data curation: CC-G DM AD DB.

Formal analysis: CC-G DM AD NK TPK DB DK WRJ AV.

Funding acquisition: WRJ AV.

Investigation: CC-G DM.

Methodology: CC-G AV DM DB DK.

Project administration: CC-G.

Resources: DB DK WRJ AV.

Software: DM DB AD.

Supervision: AV WRJ.

Validation: CC-G DM AD AV.

Writing – original draft: CC-G AD.

Writing – review & editing: DM NK TPK PJA MID RPD DB DK WRJ AV.

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Annexe 6.

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ORIGINAL ARTICLE

Impact of intraoperative blood transfusion on short and long term outcomes after curative hepatectomy for intrahepatic cholangiocarcinoma: a propensity score matching analysis by the AFC-IHCC study group

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Abstract

Background: The impact of intraoperative blood transfusion (IBT) on outcomes following intrahepatic cholangiocarcinoma (IHCC) resection remains to be ascertained.

Methods: All consecutive IHCC resected were analyzed. A first cohort (n = 569) was used for investigating short-term outcomes (morbidity and mortality). A second cohort (n = 522) excluding patients dead within 90 days of surgery was analyzed for exploring overall survival (OS) and disease free survival (DFS). Patients who received IBT were compared to those who did not, after using a propensity score matching (PSM) method.

Results: Among 569 patients, 90-day morbidity and mortality rates were 47% (n = 269) and 8% (n = 47). After PSM, 208 patients were matched. There was an association between IBT and increased overall morbidity and severe morbidity ($p = 0.010$). However, IBT did not impact 90-day mortality rate ($p > 0.999$). Regarding long-term outcomes analysis in the second cohort (n = 522), 5-year OS and DFS rates were 39% and 25%. Using PSM, 196 patients were matched and no association between IBT and OS or DFS was found ($p = 0.333$ and $p = 0.491$).

Conclusions: IBT is associated with an increased risk of morbidity but does not impact on long-term outcomes. Need for IBT should be considered as a surrogate of advanced disease requiring complex resection. Still, restricted transfusion policy should remain advocated for IHCC resection.

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Introduction

The incidence of intrahepatic cholangiocarcinoma (IHCC) has risen by 165% over the last three decades, from 0.32 per 100,000 to 0.85 per 100,000.[1,2] Complete resection is the only potential curative option but is often limited by the tumors large size, central location, and contact with major vessels including the retrohepatic inferior vena cava. Major hepatectomy is required for 51%–66% while intraoperative blood transfusion (IBT) is administered in 24%–39%[9] of patients.[3–9] Blood transfusion has been extensively identified as a risk factor for short and long term outcomes in various malignancies.[10–16] However, the relationship between IBT and outcomes remains unclear. Although IBT may have a causal effect on outcomes, one could hypothesize that IBT is a surrogate for adverse prognostic factors as recently reported in the setting of colorectal cancer patients.[17] Likewise, despite a contradictory meta-analysis,[18] Yang and colleagues demonstrated through a propensity score matching (PSM) analysis that perioperative blood transfusion did not influence survival after liver resection for hepatocellular carcinoma.[19] In the setting of IHCC, although extensive studies have investigated prognostic factors, specific data on the prognostic impact of IBT are lacking.[3–9,20,21]

CGG and AD are co-first authors and contributed equally in this work.

The above background motivated the present retrospective analysis of the French multicenter database from the IHCC-AFC Study Group to scrutinize the impact of IBT on short- and long-term outcomes after resection for IHCC. The current analysis was performed before and after matching on potential confounding prognostic factors using PSM.

Methods

Study population

Data from all consecutive patients submitted to curative-intent resection for IHCC from January 1989 to March 2009 were collected from a dedicated multi-institutional database (24 university centers) after institutional approval at each center. The present retrospective study relies on data prospectively collected for previous studies and updated at each center.[3,22] Diagnosis of IHCC relied on acknowledged criteria.[23] Patients who underwent palliative resection (R2 margin) were excluded. Two cohorts of patients further subdivided into IBT and non-IBT groups were studied: (i) a first cohort included all consecutive patients to evaluate short-term outcomes (90-day mortality and morbidity) (ii) a second cohort included all consecutive patients who survived the 90 postoperative days to analyze the impact of IBT on long-term outcomes (overall and disease free survivals, OS and DFS respectively).

Variables

Clinical preoperative variables included demographics, ASA score, underlying liver disease and preoperative tumor features, neoadjuvant treatment, and the need for portal vein embolization. Operative variables included hepatectomy extent, the need for vascular clamping and IBT. Combined vascular, biliary, or extrahepatic structures resections were also recorded, as well as lymphadenectomy whenever performed. Major and extended major resections were defined as resection of $\geq$3 or $\geq$5 segments, according to Couinaud respectively.[24]

IBT was defined as the infusion of packed red blood cells during surgery. Other blood products including fresh frozen plasma, cryoprecipitate, and platelets were rarely administered and not included in this definition. Throughout the study period, IBT was delivered to maintain the hemoglobin level >9 g/dL according to the Agence Française de Sécurité Sanitaire recommendations similar to those from the American Society of Anesthesiologists (ASA) Task Force,[25] to ensure cardiovascular status and hemodynamical stability.

Tumor pathology variables were as follows: size and number of tumors, differentiation grade, resection margin status defined as microspically incomplete (R1) or complete (R0), vascular invasion, perineural invasion, lymph node status and histology of the non-tumor liver parenchyma. Extrahepatic involvement was

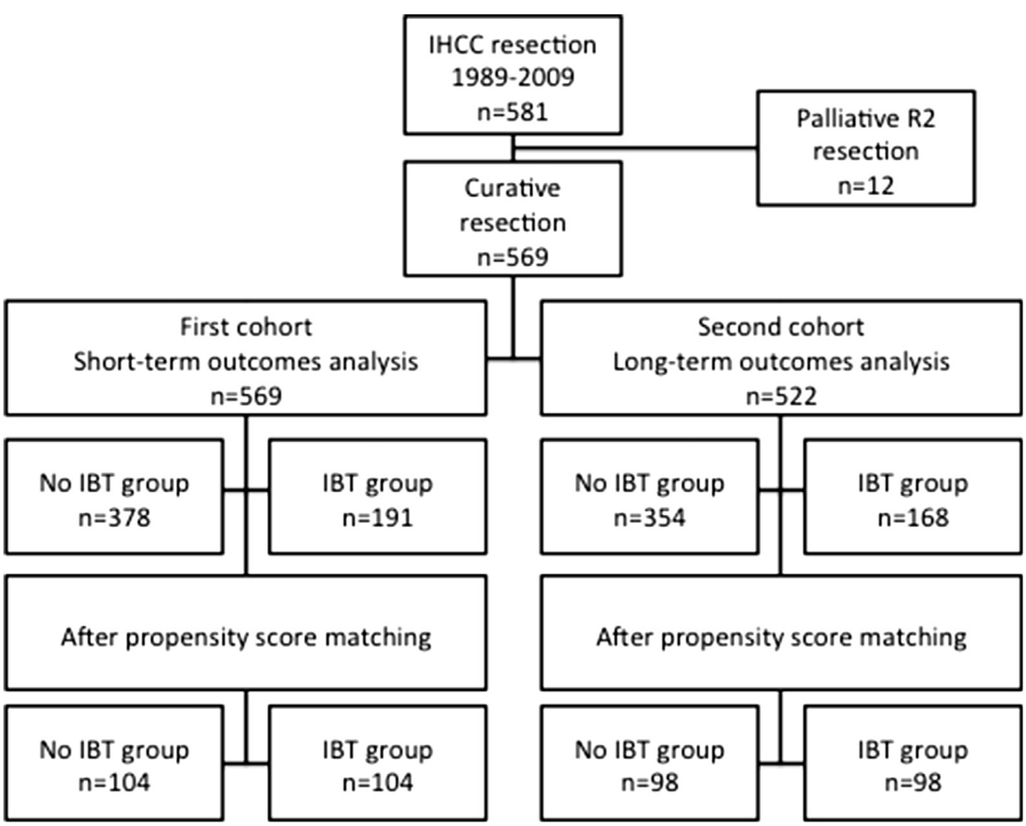


Figure 1 Study population flow chart

defined as direct invasion of any extrahepatic organs excluding the gallbladder (pT3).

Mortality and morbidity were measured within 90 days of surgery. Morbidity was graded according to the Dindo-Clavien grading system[26] and further subdivided into mild (grade I or II complications) or severe (grade III to V complications). In case of multiple complications, the highest grade was retained for analysis. Long term clinical and radiographic monitoring was performed postoperatively, every 4–6 months. Adjuvant chemotherapy consisted of gemcitabine-based regimen and was delivered at management team discretion, in case of features deemed at high risk, such as positive nodal disease, vascular invasion, or R1 resection.

Propensity score matching

Patients from the IBT and non-IBT groups were matched using the propensity score method.[27,28] The propensity score for an individual was calculated given the covariates of age, sex, body mass index (BMI), ASA score, neoadjuvant chemotherapy, preoperative portal vein embolization, resection period, hospital annual case-load, major hepatectomy, portal lymphadenectomy, vascular resection, biliary resection, associated extrahepatic resection, intraoperative vascular clamping, tumor size, multifocal disease in specimen, vascular and perineural invasion, nodal status, resection margin status and adjuvant chemotherapy. The latter was included into the model to control for the potential bias owing to the long lasting study period.

Table 1 Multivariable analysis for predictors of intraoperative blood transfusion before PSM in the first cohort (n = 569)

	OR (95% CI)	p
Patient characteristics		
Age, years	1 (0.98–1.01)	0.780
Portal Vein Embolization	2.42 (1.24–4.73)	0.009
Operative Data		
Resection period		<0.001
1989–2000	Reference	
2001–2009	0.35 (0.23–0.53)	
Hospital IHCC annual case load	1.06 (0.98–1.16)	0.121
Major Hepatectomy	1.24 (0.71–2.15)	0.430
Combined vascular resection	2.85 (1.47–5.39)	0.002
Common bile duct resection	2.15 (1.33–3.38)	0.002
Associated extrahepatic resection	1.69 (0.82–3.52)	0.147
Vascular clamping	2.62 (1.56–4.40)	<0.001
Tumor characteristics		
Tumor size, cm	1.11 (1.05–1.16)	<0.001
Multifocal disease on specimen	1.22 (0.80–1.85)	0.340
Resection margin status		0.007
R1	Reference	
R0	0.56 (0.37–0.85)	

Table 2 Characteristics of patients, tumor and outcomes in the first cohort after PSM According to intraoperative blood transfusion (n = 208)

	No-IBT n = 104	IBT n = 104	p
Variables used for matching			
Patient characteristics			
Male Gender	48 (46%)	50 (48%)	0.890
Age, years	63.4 (11.8)	64.3 (11.3)	0.600
BMI, kg/m^2	25.4 (4.5)	25.0 (4.0)	0.669
ASA Score >2	12 (11%)	9 (9%)	0.640
Portal Vein Embolization	2 (2%)	7 (7%)	0.201
Neoadjuvant Chemotherapy	9 (9%)	7 (7%)	0.790
Operative Data			
Resection period			0.650
1989–1999	33 (32%)	37 (35%)	
2000–2009	71 (68%)	67 (64%)	
Hospital IHCC annual case load	3.0 (2.1)	3.1 (2.5)	0.291
Major Hepatectomy	78 (75%)	82 (79%)	0.620
Portal lymphadenectomy	53 (51%)	56 (54%)	0.780
Combined vascular resection	5 (5%)	6 (6%)	>0.999
Common bile duct resection	20 (17%)	17 (16%)	0.710
Associated extrahepatic resection	6 (6%)	7 (7%)	>0.999
Vascular clamping	87 (84%)	82 (79%)	0.470
Tumor size, cm	7.4 (2.8)	7.1 (3.7)	0.150
Other variables			
Tumor characteristics			
Tumor size, cm	7.4 (2.8)	7.1 (3.7)	0.383
Multifocal disease on specimen	39 (37%)	43 (41%)	0.700
Vascular invasion	43 (41%)	37 (35%)	0.500
Perineural Invasion	26 (22%)	24 (23%)	0.870
Nodal status			0.761
N0	35 (35%)	34 (33%)	
N1	18 (17%)	22 (21%)	
Nx	51 (49%)	48 (46%)	
Resection margin status			0.544
R1	28 (27%)	33 (32%)	
Adjuvant Chemotherapy	36 (35%)	34 (33%)	0.880
Short-term outcomes			
Clavien I–II	17 (16%)	20 (19%)	0.710
Clavien III–IV	17 (16%)	33 (32%)	0.010
Clavien V (death)	6 (6%)	7 (7%)	>0.999
In-hospital stay, days	16.3 (14.0)	20.3 (13.5)	0.001

Data are presented as mean (standard deviation) or n (%) as appropriate. Abbreviations: No-IBT, no intraoperative transfusion; IBT, intraoperative transfusion; ASA, American Society of Anesthesiologists; BMI, Body Mass Index; PSM, propensity score matching.

A 1:1 nearest neighbor matching was performed without replacement to minimize conditional bias. For each patient performed without transfusion, a patient with intraoperative transfusion with a minimum tested in distance of propensity score was matched. Multiple caliper widths were tested. A caliper width of 0.01 resulted in the best trade-off between homogeneity and retained sample size.

Statistical analysis

Continuous variables with a normal distribution are given as mean (SD); the others are presented as median (range) and compared using the Mann–Whitney U test. Categorical variables, presented as numbers and percentages, were compared using the chi-square test for categorical variables. Univariable and multivariable regression analysis were used to identify independent predictive factors of IBT, morbidity, mortality, OS and DFS.

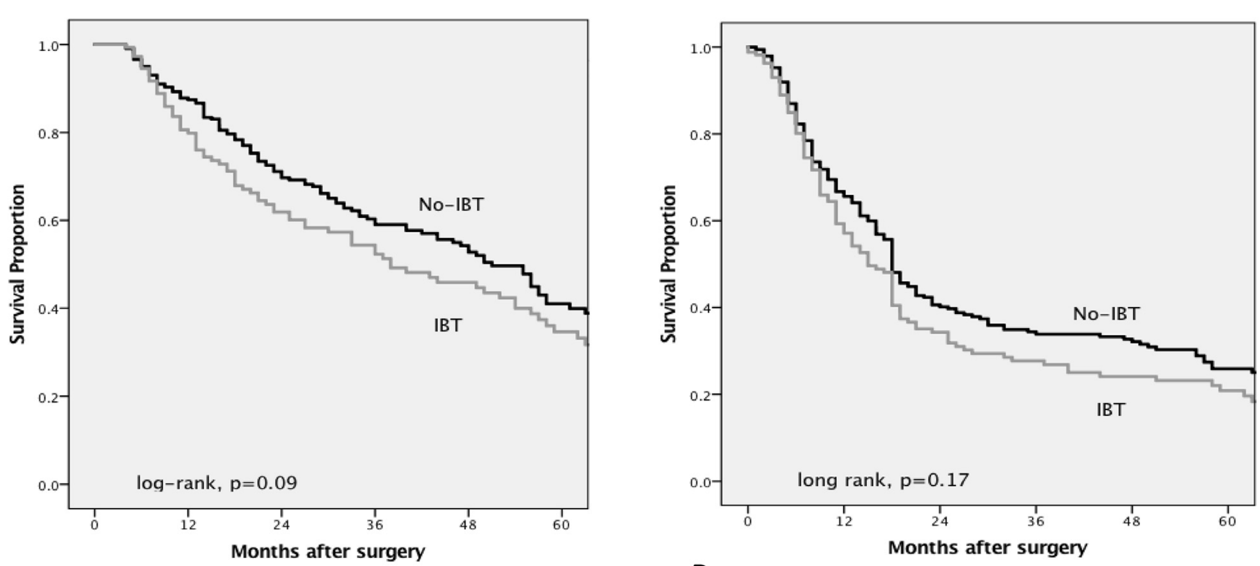
OS was calculated from the date of resection to the date of death, or the date of last follow-up and DFS was calculated from the date of resection to the date of first recurrence, the date of death or last follow-up. OS and DFS were estimated using the Kaplan–Meier method and compared between groups using the Log-rank test. p Values < 0.05 were considered statistically significant. Statistical analyses were carried out using SPSS Statistics 23.0 (IBM). The present study complied with the RECORD guidelines.[29]

Results

A total of 581 consecutive patients underwent liver resection for IHCC. Twelve patients were excluded due to palliative resection (2%). Supplemental Table 1 summarizes the main characteristics of the study population (n = 569).

First cohort analysis: short-term outcomes

The first cohort included 569 patients, of which 191 received IBT group (34%, Fig. 1). Among patients who received IBT, the amount of administered red blood packs (median = 3, range 1–37). As shown in Supplemental Table 1, patients who received IBT were significantly different in terms of demographics, resection extent and tumor features as compared to those who did not require IBT. Resection period, preoperative portal vein embolization, vascular clamping, tumor size and vascular or biliary resection were identified as independent predictors of IBT (Table 1). Overall morbidity, severe morbidity and mortality rates were 47% (n = 269), 20% (n = 113), and 8% (n = 47) respectively.



A

B

Patients at risk	Total	1 year	2 years	3 years	4 years	5 years
No IBT	354	311	209	131	88	64
IBT	168	150	100	66	50	36

Patients at risk	Total	1 year	2 year	3 year	4 year	5 years
No IBT	354	322	171	84	61	48
IBT	168	154	78	41	30	23

Figure 2 Overall (a) and disease free (b) survival curves of intraoperative blood transfusion (IBT) and no-IBT groups in the second cohort before PSM

Short-term outcomes before PSM

Overall morbidity was 64% (n = 123) in the IBT vs. 39% (n = 146) in the non IBT group (p < 0.001). Minor and major morbidity rate was significantly higher in the IBT group as compared to the non-IBT group (21% vs. 18%, p = 0.003; and 43% vs. 20%, p < 0.001, respectively, Supplemental Table 1). Ninety-day mortality occurred significantly more frequently in the IBT group as compared to the non-IBT group (13% vs. 6%, respectively, p = 0.024, Supplemental Table 1). Amount of administered red blood packs was associated with severe morbidity (RR = 1.14, 95% CI 1.04–1.25; p = 0.004) but not with increased mortality risk (RR = 1.06, 95% CI 0.99–1.12; p = 0.054).

Short-term outcomes after PSM

After PSM, 104 (54%) IBT patients could be matched with 104 (27%) non-IBT patients. All variables were balanced between the two groups (p value for standardized mean difference > 0.2). There was no significant difference in the demographic or operative characteristics between groups (Table 2). Whereas overall and severe morbidity rates remained significantly higher in the IBT as compared to the non IBT group (58% vs. 38%, and 38% vs. 22%, p = 0.010 for both comparisons), minor morbidity rate and more importantly mortality rate were similar between groups (p = 0.710, and p > 0.999 respectively, Table 2).

Second cohort analysis: long-term outcomes

The second cohort included 522 patients of which 354 were in the non-IBT group (68%), and 168 in the IBT group (32%, Fig. 1). Among patients who received IBT, the amount of administered red blood packs (median = 3, range 1–25). Median follow-up was 35 months (range 3–11). One-, 3-, and 5-year OS rates were 86, 59 and 39% respectively. Corresponding DFS rates were 67, 33 and 25%.

Long-term outcomes before PSM

Overall survival rates at 1-, 3-, and 5 years were of 82%, 56%, and 35% in the IBT group and of 88%, 61% and 42% in the non-IBT group (p = 0.094, Fig. 2A).

Similarly, there was no statistical difference between these two groups in term of DFS (62%, 29% and 22% versus 67%, 35% and 26%; p = 0.117; Fig. 2B).

On multivariable analysis, IBT was neither an independent predictor of OS (HR: 1.02 95% CI: 0.76–1.36, p = 0.891), nor of DFS (HR: 1.19 95% CI: 0.95–1.51, p = 0.710) (Supplemental Table 2 and Supplemental Table 3). Amount of IBT was not associated with OS (HR = 1.00, 95% CI 0.97–1.03; p = 0.861) and DFS (HR = 1.00, 95% CI 0.97–1.03; p = 0.971).

Long-term outcomes after PSM

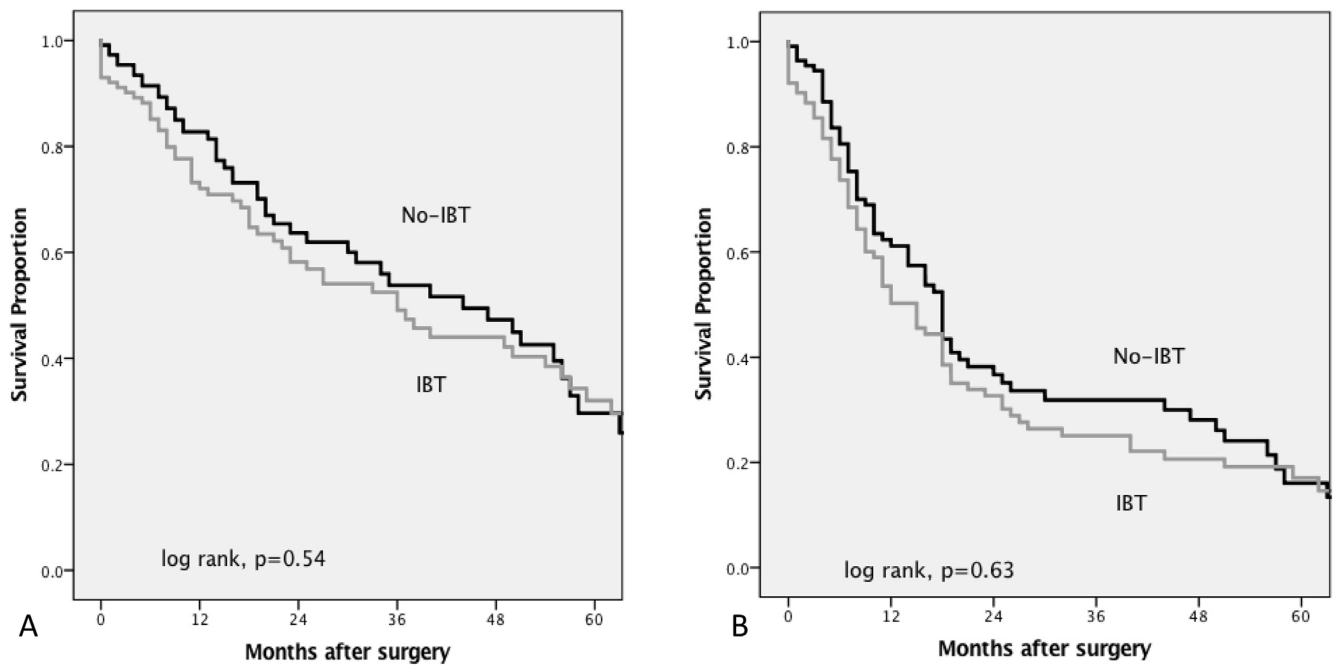
After PSM, 98 (58%) patients from the IBT group could be matched with 98 (28%) non-IBT patients (Fig. 1). All variables were balanced between the two groups (p value for standardized mean difference > 0.200). There was no significant difference in the demographic or operative characteristics between groups in both PSM (Table 3).

Table 3 Characteristics of Patients, Tumor and Outcomes of the second cohort After PSM According to Intraoperative Blood Transfusion (n = 196)

	No-IBT n = 98	IBT n = 98	p
Patient characteristics			
Male Gender	52 (53%)	48 (49%)	0.667
Age, years	64 (11.8)	64 (11.2)	0.970
BMI, kg/m^2	24.7 (3.7)	24.7 (4.2)	0.853
ASA Score >2	12 (12%)	7 (7%)	0.330
Neoadjuvant chemotherapy	6 (6%)	8 (8%)	0.780
Preoperative PVE	5 (5%)	6 (6%)	>0.999
Operative Data			
Resection period			>0.999
1989–1999	33 (40%)	34 (35%)	
2000–2009	65 (66%)	64 (65%)	
Hospital IHCC resection/year volume/year	3.1 (2.14)	3.4 (2.61)	0.840
Major Hepatectomy	81 (83%)	77 (78%)	0.581
Portal lymphadenectomy	51 (52%)	46 (47%)	0.566
Combined vascular resection	8 (8%)	8 (8%)	>0.999
Common bile duct resection	22 (22%)	17 (17%)	0.470
Associated extrahepatic resection	6 (6%)	7 (7%)	>0.999
Vascular clamping	80 (82%)	82 (84%)	0.850
Tumor characteristics			
Tumor size, cm	7.0 (3.5)	6.8 (3.4)	0.750
Multifocal disease on specimen	34 (35%)	37 (38%)	0.763
Vascular invasion	42 (43%)	39 (40%)	0.770
Perineural Invasion	24 (24%)	26 (26%)	0.870
Nodal status			0.570
N0	37 (38%)	37 (38%)	
N1	14 (14%)	39 (40%)	
Nx	47 (48%)	26 (26%)	
Resection margin status			0.750
R1	70 (71%)	67 (68%)	
Adjuvant Chemotherapy	39 (40%)	41 (42%)	0.883

Data are presented as mean (standard deviation) or n (%) as appropriate. Abbreviations: No-IBT, no intraoperative transfusion; IBT, intraoperative transfusion; ASA, American Society of Anesthesiologists; BMI, Body Mass Index; PSM, propensity score matching; PVE, portal vein embolization.

One-, 3-, and 5 years OS (Fig. 3A) was similar between IBT and non-IBT groups (83%, 57% and 32%, 85%, 57% and 40%; p = 0.333, Table 2). Likewise, 1-, 3-, and 5 years DFS (Fig. 3B) was comparable between IBT and non-IBT groups (64%, 31% and 20%, 68%, 36% and 29%; p = 0.491).



Patients at risk	Total	1 year	2 year	3 year	4 year	5 years
No IBT	98	85	53	35	21	15
IBT	98	89	60	38	30	20

Patients at risk	Total	1 year	2 year	3 year	4 year	5 years
No IBT	98	85	53	35	21	15
IBT	98	89	60	38	30	20

Figure 3 Overall (a) and disease free (b) survival curves of intraoperative blood transfusion (IBT) and no-IBT groups in the second cohort after PSM)

Univariable and multivariable Cox regression analyses of prognostic factors for OS and DFS after curative resection of IHCC in the matched cohort are shown in Tables 4 and 5, respectively.

Discussion

Complete IHCC resection is known to be complex, frequently requiring major hepatectomy and IBT. Using a robust statistical method through a large multicenter series of consecutive curative-intent hepatectomies, IBT was found to be independently associated with an increased risk of overall and severe morbidity but not with 90-day mortality. Second, IBT was not identified as an independent predictor of OS or DFS.

In the current study, IBT was delivered to approximately one third of patients, as reported in previous series (range, 17–34%).[30,31] Identified predictors of IBT (Table 2), such as vascular clamping and vascular resection are those classically reported in large series of hepatectomies and can be preoperatively anticipated.[32,33] Additionally, IBT rate significantly decreased overtime. This trend has been recently observed among large series of hepatectomies and results most likely of the continuous improvements in perioperative management, preoperative imaging/planning and surgical devices.[34,35]

Using PSM analysis allowed taking into account potential confounding factors. Thereby, IBT was not found to be an independent risk factor for 90-day mortality between two matched populations. In contrast, IBT was independently associated with increased overall and severe morbidity. Such a relationship has never been investigated in the setting of IHCC, albeit previously reported for other liver malignancies.[13,18,19] These findings suggest that IBT should be seen as an incentive for enhanced postoperative monitoring.

Regarding long-term outcomes, no impact of IBT was observed in the present study, whether before of after PSM. The current study confirms findings from previous series. However, these studies were not specifically focused on IHCC and none used a PSM method.[36,37] Until recently, IBT have been considered as having an adverse causal effect on long-term survival. Still, this dogma may be based on biased analyses from heterogeneous populations. For instance, patients who received IBT in the current study had more frequently multifocal disease, large tumor size and R1 resection margin. These features have been previously identified as adverse prognostic factors and do not

Table 4 Univariable and multivariable Cox regression analysis with robust estimator of overall survival (OS) after curative resection of intrahepatic cholangiocarcinoma after PSM in the second cohort (n = 196)

Variable	N	Univariable		Multivariable	
		p	HR (95% CI)	p	HR (95% CI)
Sex		0.920	1.01 (0.66–1.55)		
Male	88 (45%)				
Female	108 (55%)				
Age $\geq$ 70, years	71 (36%)	0.080	0.67 (1.42–1.05)	0.100	0.68 (0.43–1.08)
ASAscore > 2	17 (9%)	0.271	1.43 (0.73–2.78)		
Neoadjuvant	13 (7%)	0.763	1.13 (0.49–2.61)		
Preoperative PVE	8 (4%)	0.200	0.48 (0–55.4)		
Resection period		0.641	0.90 (0.58–1.39)		
1989–1999	64 (33%)				
2000–2009	132 (67%)				
Major hepatectomy	154 (79%)	0.070	1.58 (0.95–2.64)	0.351	1.18(0.83–1.67)
Portal lymphadenectomy	97 (50%)	0.080	1.43 (0.94–2.19)	0.001	70.2 (12.2–401)
Vascular resection	10 (5%)	0.500	1.36 (0.55–3.36)		
Biliary resection	40 (20%)	0.453	1.22 (0.71–2.11)		
Extrahepatic resection	16 (8%)	0.300	1.45 (0.70–3.02)		
Vascular clamping	168 (86%)	0.861	1.04 (0.59–1.86)		
IBT	98 (50%)	0.333	1.22 (0.80–1.87)		
Tumor size > 5 cm	55 (28%)	0.333	1.26 (0.78–2.03)		
Multifocal disease	65 (33%)	0.001	1.68 (1.10–2.57)	0.001	1.76 (1.33–2.33)
Vascular invasion	76 (39%)	0.110	1.40 (0.91–2.14)		
Perineural invasion	47 (24%)	0.461	1.19 (0.74–1.90)		
Nodal status		0.040	0.93 (0.85–1.02)	0.001	2.43 (1.67–3.54)
N0	68 (35%)				
N1	29 (15%)				
Nx	99 (50%)				
R0 Resection margin	136 (69%)	0.321	0.80 (0.51–1.25)		
Adjuvant therapy	76 (39%)	0.460	1.18 (0.75–1.86)		

Abbreviations: Non-IBT, non intraoperative transfusion; IBT, intraoperative transfusion; ASA, American Society of Anesthesiologists; BMI, Body Mass Index; PSM, propensity score matching; PVE, portal vein embolization.

need further comments.[3,31] Consequently, IBT might be better considered as a surrogate of worse survival due to aggressive tumor behavior requiring complex resection. As described for various malignancies, using PSM analysis allowed rendering patients comparable regarding established prognostic factors and shown no prognostic impact for IBT.[19,38,39]

Despite the results mentioned above, restricted transfusion policy should stand as a rule during hepatectomy for IHCC for several reasons. First, IBT is associated to increased overall and severe morbidity. Morbidity is known to involve prolonged length of hospital stay, higher postoperative mortality, and increased costs.[40] Additionally, our group among others recently showed that the occurrence of morbidity after IHCC resection independently shortened long-term survival.[22,41] Early detection is thus warranted not only as a rescue for avoiding mortality but also for preventing long-term consequences.[42] Second, appropriate delivery of blood transfusions remains of cardinal importance, as other transfusion-related adverse events are scarce but possible.[43–45]

Some limitations of the present study warrant discussion. First, patients were not randomly assigned to IBT but selected following aforementioned criteria. However, a randomized controlled trial for evaluating the impact of IBT on outcomes would be difficult to design on an ethical regard. PSM, the most robust statistical approach in the setting of a retrospective study was adopted to overcome this issue.[46] Second, this report provides complete data on a large cohort that would otherwise be difficult to accrue at a single Western center due the scarcity of

Table 5 Univariable and multivariable Cox regression analysis with robust estimator of disease free survival (DFS) after curative resection of intrahepatic cholangiocarcinoma after PSM in the second cohort (n = 196)

Variable	N	Univariable		Multivariable	
		p	HR (95% CI)	p	HR (95% CI)
Sex		0.999	1.00 (0.69–1.43)		
Male	88 (45%)				
Female	108 (55%)				
Age $\geq$ 70, years	71 (36%)	0.100	0.73 (0.50–1.07)	0.06	0.79 (0.63–1.00)
ASA score > 2	17 (9%)	0.901	1.03 (0.55–1.93)		
Neoadjuvant	13 (7%)	0.062	1.78 (0.93–3.42)	0.03	1.6 (1.04–2.47)
Preoperative PVE	8 (4%)	0.261	0.35 (0.05–2.54)		
Resection period		0.930	1.01 (0.70–1.47)		
1989–1999	64 (33%)				
2000–2009	132 (67%)				
Major hepatectomy	154 (79%)	0.011	1.7 (1.10–2.72)	0.27	1.17 (0.88–1.56)
Portal lymphadenectomy	97 (50%)	0.181	1.26 (0.88–1.81)		
Vascular resection	10 (5%)	0.530	0.75 (0.30–1.86)		
Biliary resection	40 (20%)	0.722	1.08 (0.68–1.72)		
Extrahepatic resection	16 (8%)	0.790	0.91 (0.46–1.80)		
Vascular clamping	168 (86%)	0.771	1.04 (0.59–1.86)		
IBT	98 (50%)	0.491	1.12 (0.78–1.61)		
Tumor size > 5 cm	55 (28%)	0.070	1.43 (0.95–2.16)	0.01	1.39 (1.07–1.81)
Multifocal disease	65 (33%)	0.010	1.85 (1.28–2.66)	0.001	1.99 (1.58–2.52)
Vascular invasion	76 (39%)	0.010	1.58 (1.10–2.28)	0.006	1.39 (1.10–1.76)
Perineural invasion	47 (24%)	0.160	1.32 (0.89–1.96)		
Nodal status		0.260	0.95 (0.88–1.03)		
N0	68 (35%)				
N1	29 (15%)				
Nx	99 (50%)				
R0 Resection margin	136 (69%)	0.241	0.80 (0.54–1.71)		
Adjuvant therapy	76 (39%)	0.050	1.42 (0.98–2.06)	0.18	1.17 (0.92–1.49)

Abbreviations: Non-IBT, non intraoperative transfusion; IBT, intraoperative transfusion; ASA, American Society of Anesthesiologists; BMI, Body Mass Index; PSM, propensity score matching; PVE, portal vein embolization.

IHCC. Consequently, the study time period over 20 years may implicate time lead bias especially regarding follow-up protocols and management approach that have changed overtime. Still, period of resection was included into the PSM model to control for this potential bias and did not emerge as a predictor of OS or DFS. Third, IBT only was investigated although data on peri-operative transfusion was not available for full case analysis. However, the intraoperative period is the most critical transfusion timing regarding the impact on long-term outcomes.[17] Further, the dose-dependent relationship of IBT with outcomes was not evaluated in the current study. Finally, data on surgical approach, vascular clamping type and duration were unavailable in the dataset although they might be associated with IBT.

In conclusion, IBT is associated with an increased risk of morbidity but not mortality and does not impact on long-term outcomes. Need for IBT should be considered as a surrogate of advanced disease requiring complex resection. Still, restricted transfusion policy should remain advocated during hepatectomy for IHCC.

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Conflict of interest

None declared.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.hpb.2017.01.001.

Annexe 7.

Original article

Multicentre study of the impact of morbidity on long-term survival following hepatectomy for intrahepatic cholangiocarcinoma

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Background: **The impact of morbidity on long-term outcomes following liver resection for intrahepatic cholangiocarcinoma is currently unclear.**

Methods: **This was a retrospective analysis of all consecutive patients who underwent liver resection for intrahepatic cholangiocarcinoma with curative intent in 24 university hospitals between 1989 and 2009. Severe morbidity was defined as any complication of Dindo–Clavien grade III or IV. Patients with severe morbidity were compared with those without in terms of demographics, pathology, management, morbidity, overall survival, disease-free survival and time to recurrence. Independent predictors of severe morbidity were identified by multivariable analysis.**

Results: **A total of 522 patients were enrolled. Severe morbidity occurred in 113 patients (21·6 per cent) and was an independent predictor of overall survival (hazard ratio 1·64, 95 per cent c.i. 1·21 to 2·23), as were age at resection, multifocal disease, positive lymph node status and R0 resection margin. Severe morbidity did not emerge as an independent predictor of disease-free survival. Independent predictors of time to recurrence included severe morbidity, tumour size, multifocal disease, vascular invasion and R0 resection margin. Major hepatectomy and intraoperative transfusion were independent predictors of severe morbidity.**

Conclusion: **Severe morbidity adversely affects overall survival following liver resection for intrahepatic cholangiocarcinoma.**

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Introduction

The incidence of intrahepatic cholangiocarcinoma (IHCC) has risen from 0·32 to 0·85 per 100 000 over recent decades[1]. Complete resection is the only potentially curative treatment, yielding a 5-year overall survival (OS) rate ranging from 21 to 35 per cent and a median OS of up to 39 months[2–4]. Prognostic models of OS and disease-free survival (DFS) rely mostly on tumour features[5–7]. Morbidity following liver resection for IHCC ranges from 35 to 45 per cent[8,9], and is associated with prolonged length of hospital stay, postoperative mortality and increased costs[10]. Postoperative morbidity has been identified as an independent predictor of worse long-term outcomes in various cancers[11–13], including after liver resection for colorectal cancer liver metastases (CRLM)[14,15] and hepatocellular carcinoma (HCC)[16–18]. Only one study[9] has reported this relationship in the setting of IHCC[9]. Confirmation of this relationship between morbidity and long-term outcomes following surgery for IHCC could affect the management of such patients, from selection for surgery to the postoperative strategy of follow-up and adjuvant treatment.

The present analysis of the French multicentre database of the Association Française de Chirurgie (AFC) IHCC study group had the primary objective of evaluating the impact of severe morbidity on long-term outcomes after liver resection with curative intent for IHCC in a large cohort. The secondary objective was to identify independent predictors of severe postoperative complications.

Methods

Data on all consecutive patients undergoing resection with curative intent for IHCC from January 1989 to March 2009 were collected from a dedicated multi-institutional database containing data from 24 university hospitals. Details of the methodology have been reported previously[3,19]. Briefly, data were collected and updated by retrospective review of medical records obtained with institutional approval at each centre. The diagnosis of IHCC was confirmed by immunohistochemistry. Mixed cholangiocarcinoma–HCCs and cholangiocarcinomas arising from the perihilar biliary tract or gallbladder were excluded[20]. Patients who underwent palliative resection (R2 resection or associated resection of synchronous peritoneal carcinomatosis) and those who died within 90 days of surgery were excluded from the analysis exploring the impact of morbidity on long-term outcomes[21].

Data collection

Preoperative variables included demographics, ASA grade, underlying liver disease and preoperative tumour features. Each specimen was subjected to the following pathological analyses: size and number of tumours, differentiation grade, resection margin status, vascular invasion, perineural invasion, lymph node status and histology of the non-tumoral liver parenchyma. Morphological subtypes were defined as mass-forming, periductal infiltrating and mixed subtypes[22]. Extrahepatic involvement and tumour stage were assessed according to the seventh edition of the AJCC staging system[7].

Operative variables included the need for vascular clamping, intraoperative blood transfusion and duration of operation. Major resection was defined as resection of at least three Couinaud segments[23]. Combined resections of vascular, biliary or extrahepatic structures were also recorded, as well as whether lymphadenectomy was performed. The time interval during which hepatectomy was undertaken and volume of liver resection for IHCC per hospital were also included in the morbidity analysis.

Long-term clinical and radiological monitoring was performed every 4–6 months, following each institutional protocol. Treatment by adjuvant chemotherapy was at the discretion of each centre in the event of lymph node-positive disease, vascular invasion or R1 resection.

Definitions of postoperative morbidity

Any postoperative event occurring within 90 days and deemed as leading to deviation from the normal postoperative course was considered a complication. Abdominal complications were dichotomized into hepatic and non-hepatic. The former included postoperative liver failure, biliary fistula and haemorrhage, according to the definitions of the International Study Group of Liver Surgery[24–26], cholangitis defined as fever and leucocytosis requiring antibiotics or biliary drainage[27], and vascular thrombosis. Abdominal non-hepatic complications included postoperative ileus, gastroparesis, intra-abdominal infection, gastrointestinal bleeding and wound dehiscence/infection. Non-abdominal complications comprised pulmonary complications (pneumonia, pleural effusion, respiratory insufficiency, pulmonary embolism), urinary complications (urinary tract infections, urinary retention) and other types, including cardiac complications, deep vein thrombosis, acute renal failure and catheter-related infections.

Morbidity was graded according to the Dindo–Clavien classification[28]. Severe morbidity was defined as any complication graded III or IV. In patients with multiple complications, the highest grade was retained for analysis. Patients experiencing severe morbidity were compared with those without severe morbidity (grade 0–II) and with patients experiencing minor morbidity (grade I–II).

Statistical analysis

The χ^2 test was used for analysis of categorical variables. Continuous variables with a normal distribution are presented as mean(s.d.) and non-normally distributed variables as median (range); *t* test and Mann–Whitney *U* test respectively were used for statistical analysis.

Time to recurrence was measured from date of resection to date of first imaging showing recurrence or date of last follow-up in the absence of recurrence. OS corresponded to the interval between date of primary resection and date of last follow-up or death. DFS was the interval between date of primary resection and date of last follow-up, death or recurrence. OS, DFS, time to recurrence and OS from the time of recurrence were estimated using the Kaplan–Meier method. Survival differences between groups were compared using the log rank test. All perioperative variables associated with survival in univariable analysis ($P < 0{\cdot}200$) were included in a Cox proportional hazard model in order to identify independent prognostic predictors. AJCC stage was not included in survival analyses to avoid collinearity with its different components (tumour size, number of nodules, vascular invasion

Table 1 Demographics and perioperative variables

	AFC cohort (*n* = 522)	Dindo–Clavien grade 0–II (*n* = 409)	Dindo–Clavien grade III–IV (*n* = 113)	*P*‡
Age (years)*	64·0 (11·7)	63·9 (11·8)	64·3 (11·3)	0·841§
Sex ratio (M : F)	254 : 268	197 : 212	57 : 56	0·520
BMI (kg/m²)*	25·6 (4·3)	25·5 (4·3)	26·0 (4·2)	0·338§
ASA grade > II	51 (9·8)	37 (9·0)	14 (12·4)	0·411
Viral hepatitis	32 (6·1)	27 (6·6)	5 (4·4)	0·383
Cirrhosis	27 (5·2)	25 (6·1)	2 (1·8)	0·090
Preoperative tumour size (cm)*	6·8 (3·8)	6·6 (3·8)	7·0 (3·8)	0·001§
Multifocal disease on imaging	79 (15·1)	59 (14·4)	20 (17·7)	0·391
Vascular involvement on imaging	75 (14·4)	50 (12·2)	25 (22·1)	0·028
Preoperative CA19-9 (units/ml)†	50·5 (0–75 000)	37 (0–75 000)	69 (0–12 082)	0·149¶
Neoadjuvant therapy	34 (6·5)	26 (6·4)	8 (7·1)	0·779
Preoperative PVE	39 (7·5)	30 (7·3)	9 (8·0)	0·864
Resection period				0·831
1989–1994	33 (6·3)	24 (5·9)	9 (8·0)	
1995–1999	87 (16·7)	67 (16·4)	20 (17·7)	
2000–2004	185 (35·4)	147 (35·9)	38 (33·6)	
2005–2009	217 (41·6)	171 (41·8)	46 (40·7)	
Hospital IHCC resection volume/year	2·6 (2·3)	2·6 (2·3)	2·6 (2·2)	0·778
Major hepatectomy	401 (76·8)	301 (73·6)	100 (88·5)	0·001
Portal lymphadenectomy	276 (52·9)	209 (51·1)	67 (59·3)	0·140
Combined vascular resection	40 (7·7)	29 (7·1)	11 (9·7)	0·339
Common bile duct resection	90 (17·2)	67 (16·4)	23 (20·4)	0·352
Associated *en bloc* extrahepatic resection	34 (6·5)	25 (6·1)	9 (8·0)	0·490
Intraoperative transfusion	168 (32·2)	109 (26·7)	59 (52·2)	< 0·001
Adjuvant therapy	178 (34·1)	146 (35·7)	32 (28·3)	0·153

Values in parentheses are percentages unless indicated otherwise; *values are mean(s.d.) and †median (range). AFC, Association Française de Chirurgie; CA, carbohydrate antigen; PVE, portal vein embolization; IHCC, intrahepatic cholangiocarcinoma. ‡χ^2 test, except §*t* test and ¶Mann–Whitney *U* test.

and nodal status). Backward selection was used, with a 0·1 cut-off for entry into the model. Independent predictors of postoperative morbidity were identified by means of a multiple logistic regression model.

All *P* values were based on two-tailed statistical analysis and $P < 0{\cdot}050$ was considered to indicate statistical significance. Analyses were performed with SPSS® software, version 22.0 for Windows® (IBM, Armonk, New York, USA). The present study complied with the RECORD guidelines[29].

Results

During the study interval, 581 consecutive patients underwent liver resection for IHCC in 24 centres. Overall, 59 patients (10·2 per cent) were excluded: 12 following palliative resection (2·1 per cent) and 47 who died within 90 days of surgery (8·1 per cent). The final study population included 522 patients (*Table 1*).

Major hepatectomy was performed in 401 patients (76·8 per cent), including 155 extended liver resections (29·7 per cent). Some form of vascular clamping was applied in 398 patients (76·2 per cent). Combined extrahepatic resections were undertaken in 138 patients (26·4 per cent) with a median of 1 (range 1–3) per patient. Biliary resection

Table 2 Tumour features

	No. of patients (*n* = 522)
Morphological type	
Mass-forming	367 (70·3)
Periductal invasion	9 (1·7)
Intraductal growth	6 (1·1)
Mixed type	58 (11·2)
Unknown	82 (15·7)
Multifocal disease	187 (35·8)
Tumour size (cm)*	7·1(4·0)
Vascular invasion	201 (38·5)
Perineural invasion	124 (23·8)
pN category	
pN0	191 (36·6)
pN1	85 (16·3)
pNx	246 (47·1)
Margin status	
R0	365 (69·9)
R1	157 (30·1)
Underlying liver parenchyma	
Cirrhosis	25 (4·8)
Steatosis	142 (27·2)
AJCC stage (7th edition)	
I	201 (38·5)
II	202 (38·7)
III	27 (5·2)
IV	92 (17·6)

Values in parentheses are percentages unless indicated otherwise; *values are mean(s.d.).

Table 3 Cox regression analysis of prognostic factors associated with overall and disease-free survival

	Overall survival		Disease-free survival	
	Hazard ratio	*P*	Hazard ratio	*P*
Age (per year)	0·988 (0·978, 0·999)	0·033	0·991 (0·981, 0·999)	0·042
Transfusion			1·05 (0·82, 1·34)	0·710
Severe morbidity	1·64 (1·21, 2·23)	0·002	1·15 (0·88, 1·50)	0·310
Tumour size (per cm)	1·02 (0·98, 1·06)	0·330	1·04 (1·01, 1·07)	0·006
Multifocal disease	1·78 (1·34, 2·36)	< 0·001	1·74 (1·36, 2·23)	< 0·001
Vascular invasion	1·31 (0·97, 1·77)	0·078	1·37 (1·09, 1·74)	0·008
Nodal status				
pN0	1·00 (reference)	< 0·001	1·00 (reference)	0·016
pN1	2·49 (1·71, 3·64)	< 0·001	1·58 (1·15, 2·17)	0·005
pNx	1·21 (0·88, 1·66)	0·250	1·09 (0·85, 1·41)	0·491
R0 margin status	0·62 (0·47, 0·83)	0·001	0·74 (0·58, 0·94)	0·016

Values in parentheses are 95 per cent confidence intervals.

was carried out in 90 patients (17·2 per cent), and combined vascular resection in 40 (7·7 per cent) (inferior vena cava, 23; portal vein, 18; hepatic artery, 10; multiple resections, 18). *En bloc* resection of extrahepatic structures was performed in 34 patients (6·5 per cent), including the diaphragm (23), right adrenal gland (4), abdominal wall (3), stomach (3) and right colon (1). Intraoperative blood transfusion was required in 168 patients (32·2 per cent). A complete resection (R0) was achieved in 365 patients (69·9 per cent). Portal lymphadenectomy was carried out in 276 patients (52·9 per cent), of whom 85 (30·8 per cent) had positive nodes (*Table 2*). Median duration of surgery was 280 (range 100–720) min. After operation, 178 patients (34·1 per cent) received adjuvant therapy, mostly consisting of systemic chemotherapy alone (154).

Postoperative morbidity

Overall, 222 patients experienced complications (morbidity rate 42·5 per cent). The minor morbidity rate was 20·9 per cent (109 patients), and the major morbidity rate 21·6 per cent (113 patients) (*Table S1*, supporting information). Among these 222 patients, 55 had multiple complications (10·5 per cent of all 522 patients), with a median of 2 (range 2–5) per patient. The overall median duration of hospital stay was 14 (range 3–122) days. Hospital stay was significantly longer among patients who developed major complications: 29 (8–122) days *versus* 12 (range 3–27) days among those with grade 0–II complications ($P < 0·001$). The time period of resection and case volume per centre were not associated with the occurrence of severe morbidity ($P = 0·831$ and $P = 0·778$ respectively). Patients experiencing severe morbidity were not less likely to receive adjuvant chemotherapy ($P = 0·153$).

Perioperative variables associated with severe morbidity in univariable analysis are shown in *Table 1*. In a multiple logistic regression model, major hepatectomy (risk ratio (RR) 2·21, 95 per cent c.i. 1·17 to 4·17; $P = 0·015$) and intraoperative transfusion (RR 2·77, 1·77 to 4·34; $P < 0·001$) were identified as independent predictors of severe morbidity.

Impact of morbidity on overall and disease-free survival

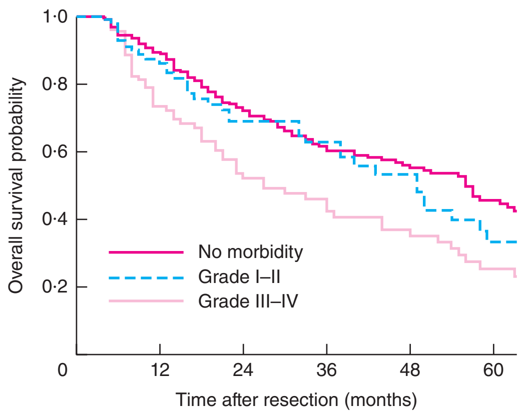
Median follow-up was 35 (range 3–211) months. The 1-, 3- and 5-year OS rates were 86·1, 59·3 and 39·4 per cent respectively. Corresponding DFS rates were 66·8, 33·4 and 25·2 per cent.

Severe morbidity was an independent predictor of worse OS in the full cohort (522 patients) (*Table 3*). Although significant on univariable analysis, severe morbidity was not independently associated with DFS.

Similar results were obtained after exclusion of patients with an uneventful postoperative outcome (300 with Dindo–Clavien grade 0). Median OS was shorter among those with severe morbidity (grade III–IV): 27 (range 16–38) months *versus* 49 (38–60) months for those with grade I–II complications ($P = 0·011$) (*Fig. 1*). The occurrence of severe morbidity was not associated with DFS: 18 (16–21) *versus* 17 (14–20) months for grade I–II *versus* III–IV ($P = 0·860$) (*Fig. 2*).

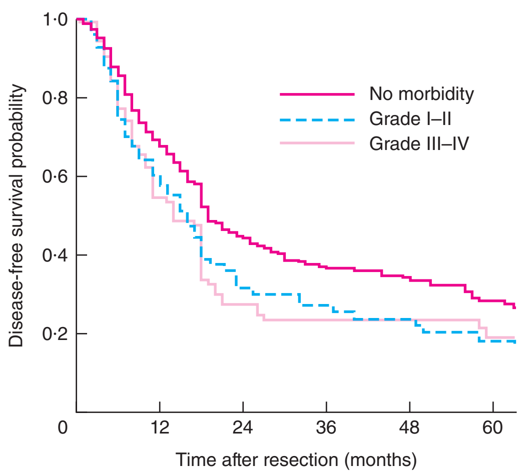
Association between severe morbidity and time to recurrence

Overall, 248 patients (47·5 per cent) experienced tumour recurrence within a median of 10 (range 1–101) months; 89·9 per cent of recurrences (223 patients) occurred within 24 months of surgery. Median time to recurrence was



No. at risk						
No morbidity	300	269	193	127	87	63
Grade I–II	109	91	57	35	26	18
Grade III–IV	113	101	59	34	25	18

Fig. 1 Kaplan–Meier estimates of overall survival for patients stratified by morbidity grade. $P = 0{\cdot}004$ (overall), $P = 0{\cdot}011$ (grade I–II *versus* III–IV), $P = 0{\cdot}001$ (no morbidity *versus* grade III–IV) (log rank test)



No. at risk						
No morbidity	300	277	159	86	62	47
Grade I–II	109	96	44	20	16	12
Grade III–IV	113	103	46	18	13	11

Fig. 2 Kaplan–Meier estimates of disease-free survival for patients stratified by morbidity grade. $P = 0{\cdot}027$ (overall), $P = 0{\cdot}860$ (grade I–II *versus* III–IV), $P = 0{\cdot}020$ (no morbidity *versus* grade III–IV) (log rank test)

shorter in patients who experienced grade III–IV morbidity compared with those with grade 0–II morbidity (18 *versus* 21 months; $P = 0{\cdot}029$). Cox regression analysis identified severe morbidity, tumour size, multifocal disease, vascular invasion and R0 resection as independent predictors of time to recurrence (*Table S2*, supporting information).

Among these 248 patients, median OS from the time of diagnosis of recurrence was 16 (95 per cent c.i. 13 to 19) months, with OS rates at 1, 3 and 5 years of 60·3, 15·6 and 3·1 per cent respectively (*Fig. S1*, supporting information). OS after recurrence was associated with severe morbidity (hazard ratio 6·11; $P = 0{\cdot}013$).

Discussion

In the present multicentre study, severe morbidity following liver resection for IHCC was an independent predictor of poor OS. Severe morbidity was also independently associated with a shorter time to recurrence, whereas this was not the case for DFS. The incidence of severe complications, OS and DFS were not associated with hospital volume, nor the time period in which the resections were carried out.

Postoperative complications occurred over 40 per cent of patients, of which half were graded as severe. These results are consistent with previously published series[4,8,9]. In the present study, OS was shorter if severe morbidity occurred. An adverse impact of morbidity on survival in the setting of IHCC was reported recently, with a trend towards worse outcomes as complication grade increased[9]. The present study has confirmed the influence of severe morbidity. Such an impact of postoperative outcomes on survival has already been reported in the field of CRLM and HCC (*Table S3*, supporting information). In the setting of CRLM, several series also reported that patients with major complications had shorter survival than those with minor complications[14,15].

Several potential confounders were taken into account in this study. Age and tumour features were identified as independent prognostic factors for OS. Surprisingly[6], younger age was an adverse prognostic factor. It could be hypothesized that tumours behave more aggressively in young patients[30,31]. Indeed, in the present cohort, younger patients (aged below 50 years) were less likely to harbour early pT1 disease. Additionally, younger patients were more likely to undergo extended liver resection combined with extrahepatic resection and to receive adjuvant therapy. Tumour features such as multifocal disease, positive lymph node status and negative resection margins were identified as independent predictors of OS in the present study, in keeping with several previous large series[2–5,7].

Age and tumour factors (size, vascular invasion, multifocal disease, positive nodal disease and negative resection margin) were independent predictors of OS and/or DFS, whereas severe morbidity was not independently associated with DFS. These findings are in accordance with those of other series[7,32], and suggest that tumour recurrence is driven mainly by tumour features. However, median time to recurrence was shorter in patients who experienced grade III–IV morbidity compared with those who had minor morbidity. Furthermore, the Kaplan–Meier survival curves showed that the relative reduction in survival associated with severe morbidity occurred early on, with survival curves diverging within 12 months after surgery (*Fig. 1*). This observation, added to the reduced time to recurrence among patients with severe morbidity, confirms earlier data demonstrating the impact of severe anastomotic leak on OS and DFS after resection for oesophageal cancer[11]. It could be hypothesized that recurrence is inherent to tumour features, but occurs earlier in the event of severe morbidity.

Major hepatectomy and intraoperative blood transfusion were found to be independent predictors of severe morbidity. As in other large series[33], these events were common in the present cohort: over 75 per cent of patients underwent major resection during which more than one-third received a transfusion. New strategies are still needed towards hepatic parenchymal sparing and blood loss reduction.

Tumour features are considered as the main determinant of survival. Based on this, it could be argued that severe morbidity is a surrogate for advanced disease requiring major resection and intraoperative blood transfusion, which is consequently associated with poor prognosis. However, severe morbidity was independently associated with worse OS. This suggests that long-term survival may be worsened by the occurrence of severe morbidity, whatever the disease stage or whether recurrence develops.

Identification of risk factors for severe morbidity are key to improving information and selection of patients with IHCC as potential candidates for surgery. In addition to reassessment of prognosis in patients with severe postoperative morbidity, strategies directed at early detection of complications before they become severe could improve long-term prognosis. This proposal is parallel to the recent concept of ability to rescue to prevent death after major complications[34]. The survival benefit provided by adjuvant treatment for patients with high-risk tumour features remains to be ascertained for those with severe morbidity. Postoperative screening for recurrence might be adapted to the occurrence of postoperative severe complications for these patients with a shorter time to recurrence.

In addition to its retrospective nature, some limitations of the present study warrant discussion. The study time period may implicate an effect of time lead bias, especially regarding changes in follow-up protocols and management approach over time. However, time and centre were not associated with morbidity or survival. In addition, this report provides complete data on a large cohort that would otherwise be difficult to accrue at a single Western centre owing the rarity of IHCC. Another limitation is that data on surgical approach, and vascular clamping type and duration were not available in the data set, and they might be associated with morbidity and long-term outcomes.

Collaborators

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Disclosure

The authors declare no conflict of interest.

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Supporting information

Additional supporting information may be found in the online version of this article:

Table S1 Postoperative morbidity (Word document)

Table S2 Cox regression analysis identifying independent predictors of time to recurrence (Word document)

Table S3 Published studies regarding the influence of morbidity on long-term survival after liver resection for hepatic malignancies (Word document)

Fig. S1 Impact of morbidity grade on overall survival after diagnosis of recurrence (Word document)

PRONOSTIC DU CHOLANGIOCARCINOME INTRAHEPATIQUE RÉSÉQUÉ

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Introduction. Alors qu'elle constitue le seul traitement curatif du cholangiocarcinome intrahépatique (CCIH), la résection reste associée à un taux de récidive supérieur à 60% et un taux de survie réelle à 5 ans inférieur à 20%. Une estimation fiable du pronostic ainsi qu'une meilleure compréhension de la biologie tumorale est essentielle pour améliorer le pronostic.

Méthodes. A l'appui des données clinico-biologiques de deux larges cohortes de patients avec CCIH réséqué (MSKCC, n=189 et AFC, n=522), trois objectifs ont été explorés. D'abord, définir quel modèle pronostique est le plus performant. Ensuite, définir la fiabilité de l'évaluation pronostique préopératoire à partir de, respectivement, l'imagerie, des microARN (miR) circulants diagnostiques et du profil génomique tumoral. Enfin, évaluer l'impact pronostique d'événements périopératoires tels que transfusion et morbidité.

Résultats. Premièrement, les nomogrammes apportaient une meilleure estimation pronostique en comparaison à la classification AJCC 7ème édition. Deuxièmement, la taille et la multifocalité tumorale sur l'imagerie préopératoire permettaient de différencier deux groupes de patients de pronostic clairement distincts ($p<0{,}001$). L'existence d'une mutation d'un gène de remodelage de la chromatine (BAP1, ARID1A, PBRM1) tendait à être associé à une survie sans récidive plus favorable qu'en l'absence de mutation ($p=0{,}09$). Alors qu'ayant un potentiel comme marqueur diagnostique circulant, miR21 et miR221 n'étaient pas associé à la survie. Troisièmement, la transfusion peropératoire n'impactait pas la survie à long terme alors que la survenue d'une complication sévère (grade Dindo-Clavien > 2) était indépendamment associée à une survie globale plus courte ($p=0{,}002$).

Conclusion. Alors que les nomogrammes postopératoires apportent une meilleure estimation pronostique, le développement de modèles pronostiques préopératoires est faisable notamment à partir de l'imagerie et de marqueurs biologiques tumoraux complémentaires.

Mots-clés : cholangiocarcinome intrahépatique ; résection ; survie ; modèles pronostiques ; génomique ; microARN circulant