Growth patterns of colorectal cancer liver metastases and their impact on prognosis: a systematic review

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Supplementary Data

Supplementary Figure 1: Search strategy for MEDLINE

Supplementary Figure 2: Search strategy for Embase

Supplementary Figure 3: Risk of Bias assessments. a) Review authors' assessment of the likelihood of bias (percentage of all included studies); "n/a": not applicable (studies that did not assess clinical outcome). **b)** Risk of Bias presented on a per study basis.

Supplementary Table 1: Histopathological definitions in eligible studies

Supplementary Table 2: Outcome analysis in eligible studies that assessed at least one outcome item

Supplementary Figure 1: Medline(Ovid) strings

Field labels:

.ti,ab,kf. =	title, abstract, keyword
exp/ =	MeSH, exploded
/ =	MeSH, not exploded
adjx =	within x number of words

1. exp Colorectal Neoplasms/
2. ((colon or colorectal or rectal) adj1 (cancer* or neoplasm* or tumor* or tumour* or carcinoma*)).ti,ab,kf.
3. or/1-2

4. exp Neoplasm Metastasis/
5. exp Liver Neoplasms/
6. Neoplasm Recurrence, Local/
7. ((liver* or hepat*) adj1 (metasta* or pathol* or second*)).ti,ab,kf.
8. or/4-7

9. Neovascularization, Pathologic/
10. (angiogen* or neovascularizat* or neovascularisat* or vascularizat* or vascularisat*).ti,ab,kf.
11. (growth pattern* or invasion front* or infiltrativ* or encapsulat* or desmoplastic* or pseudocapsule* or
pushing or replacement*).ti,ab,kf.
12. or/9-11

13. 3 and 8 and 12
14. 13 not (animals not humans).sh.
15. remove duplicates from 14

Supplementary Figure 2: Embase (embase.com) strings

:ti,ab = title, abstract
exp/ = Emtree, exploded
de/ = Emtree, non exploded
NEAR/1 = within one word

#1 'colorectal tumor'/exp
#2 ((colon OR colorectal OR rectal) NEAR/1 (cancer* OR neoplasm* OR tumor* OR tumour* OR carcinoma*)):ab,ti
#3 #1 OR #2

#4 'metastasis'/exp
#5 'liver tumor'/exp
#6 'tumor recurrence'/de
#7 ((liver* OR hepat*) NEAR/1 (metasta* OR pathol* OR second*)):ti,ab
#8 #4 OR #5 OR #6 OR #7

#9 'neovascularization (pathology)'/de
#10 angiogen*:ti,ab OR neovascularizat*:ti,ab OR neovascularisat*:ti,ab OR vascularizat*:ti,ab OR vascularisat*:ti,ab
#11 growth:ti,ab AND pattern*:ti,ab OR invasion:ti,ab AND front*:ti,ab OR infiltrativ*:ti,ab OR encapsulat*:ti,ab OR desmoplastic*:ti,ab OR pseudocapsule*:ti,ab OR pushing:ti,ab OR replacement*:ti,ab
#12 #9 OR #10 OR #11

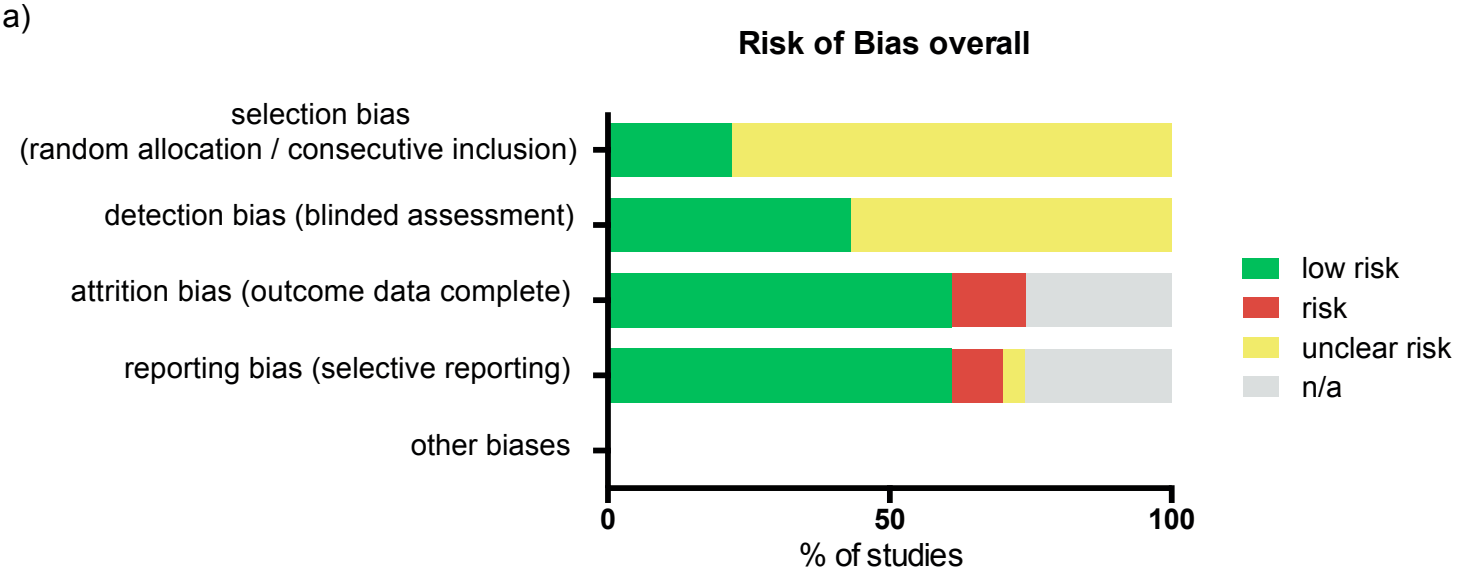
#13 #3 AND #8 AND #12
#14 [animals]/lim NOT [humans]/lim
#15 #13 NOT #14

#16 #15 AND ('Conference Abstract'/it OR 'Conference Paper'/it OR 'Conference Review'/it)

#17 #15 NOT #16

Supplementary Figure 3

a)



b)

Risk of Bias per individual study

	selection bias (random allocation /consecutive inclusion)	detection bias (blinded assessment)	attrition bias (outcome data complete)	reporting bias (selective reporting)
Dam 2017	?	?	+	+
Frentzas 2016	?	+	+	+
Siriwardana 2016	?	+	+	?
Serrablo 2016	+	+	+	+
Eefsen 2015a	+	+	n/a	n/a
Brunner 2014	?	+	-	+
Pinheiro 2014	?	+	+	+
Wiggans 2012	?	+	+	+
Nyström 2012	?	?	+	+
Van Den Eynden 2012	?	+	+	+
Rajaganeshan 2007a	?	?	+	+
Stessels 2004	?	?	n/a	n/a
Terayama 2002	?	?	n/a	n/a
Yamaguchi 2002	?	?	-	-
Vermeulen 2001	?	?	n/a	n/a
Weber 2001	?	?	+	+
Lunevicius 2001	?	?	+	-
Okano 2000	+	+	+	+
Ambiru 1999	?	?	-	+
Nagashima 1999	+	+	+	+
Terayama 1996	?	?	n/a	n/a
Yamamoto 1995	+	?	n/a	n/a
Morino 1991	?	?	+	+

Supplementary Table 1: Histopathological definitions in the eligible studies.

study	desmoplastic type	pushing type	replacement type
Dam 2017	"Desmoplastic rim between liver and metastatic tissue"	"Liver pushed aside by metastatic tissue"	"Hepatocytes are replaced by cancer cells"
	Note that in addition to these definitions, Dam et al. provide concise guidelines and a flow diagram describing in detail the suggested scoring procedure.		
Frentzas 2016, London and Montréal cohort	"no direct contact between cancer cells and liver parenchyma [...] and the cancer cells [are] separated from the liver parenchyma by a layer of desmoplastic stroma"	"close contact between cancer cells and normal liver tissue [...] without an intervening desmoplastic stroma; The normal liver [is] compressed by the tumor and no invasion of cancer cells into the hepatic plates [is] observed" Note: Assigned cases showing areas of direct replacement next to a pushing phenotype to the replacement pattern.	"close contact between cancer cells and liver parenchyma [is] observed, without an intervening desmoplastic stroma. Cancer cells [have] invaded the hepatic plates and [replace] the hepatocytes without destroying the vascular architecture of the liver at the tumor–liver interface"
Siriwardana 2016	"a fibrotic or desmoplastic layer between the metastasis and the liver parenchyma. There [is] a lymphocytic infiltrate and bile ductules within the layer of desmoplasia. The tumor margin [is] intact. The glands [are] large, complex, and cribriform"	n/a	"the metastasis [is] infiltrating the liver sinusoids with no desmoplasia or lymphocytic infiltrate in the CRLM-liver interface. The glands [are] small, closely packed, individually arranged, and rounded..."
Serrablo 2016	"defined according to the Lunevicius criterion, as the presence of a perilesional fibrous reaction >= 0.5 mm around the entire contour of the lesion."	"The factor 'tumour growth type' (infiltrative or expansive) has previously been described by our group, and hypoxic or non hypoxic were evaluated as two different 'growth patterns' "	The factor "tumour growth type" (infiltrative or expansive) has previously been described by our group, and hypoxic or non hypoxic were evaluated as two different "growth patterns"
Eefsen 2015a	"characterised with a collagen rim surrounding the metastatic tumour cells"	"expansive growth of the tumour cell and flattening surrounding hepatocytes"	"characterised by an infiltrative growth where tumour and hepatocytes grow side by side."
Brunner 2014	presence of a "fibrotic capsule"		
Pinheiro 2014	n/a	"the tumor's edges expanded, pushing the adjacent liver tissue reasonably well circumscribed or involved by a thin capsule."	"'when the tumor spread freely through the surrounding tissue, dissecting between normal hepatocytes in an effortless fashion, usually unoppsoed by any form of inflammatory host response"
Wiggans 2012	"paucicellular collagenous band present between the tumour cells and the adjacent hepatocytes, which measured at least 0.1 mm in thickness."	n/a	n/a
Nyström 2012	"The expression intensity and the patterns of stromal collagens were graded as 0=no expression, 1=mild to moderate expression, and 2=strong expression. The expression intensity in CRC was graded both in the desmoplastic reaction (DR) and in the immediate vicinity of the cancer cell"	"Tumour cells were separated from the normal liver parenchyma by merely a thin rim of collagen and with only a mild inflammatory infiltrate present. "	n/a
Van Den Eynden 2012	"...the tumour was separated from the liver parenchyma by a layer of desmoplastic stroma infiltrated with lymphocytes and nests of tumour cells. There was no direct contact between tumour cells and liver parenchyma."	"the liver plates [are] compressed, running parallel to the tumour liver interface without desmoplastic stroma and only a mild inflammatory infiltrate. "	"In the replacement pattern tumour cells and liver parenchyma [are] in close approximation with no compression of the plates, no desmoplastic stroma or inflammatory infiltrate; the tumour cells [replace] the hepatocytes in the liver cell plates without destruction of the liver architecture"
Rajaganeshan 2007a	"The invasive margin was classified using a modified classification based on [Lunevicius] et al. The metastases were classified as capsulated if >50% of the margin exhibited a fibrous capsule separating tumour from stroma."	n/a	n/a
Stessels 2004	"...the metastases were separated from the surrounding liver parenchyma by a rim of desmoplastic stroma in which a dense mononuclear infiltrate and numerous capillaries were present. Often tumour cell nests were infiltrating the stroma. The tissue architecture of the liver was not conserved within the metastases. "	"In the 'pushing' growth pattern, liver plates [are] pushed aside and [run] in parallel with the circumference of the metastases at the tumour – liver parenchyma interface. There [is] no desmoplastic stroma formation, and the tumour cells [are] separated from the hepatocytes by a thin layer of connective tissue fibres. A mild inflammatory infiltrate [is] nearly always present at the interface. The tissue architecture of the liver [is] not conserved within the metastases.	"... tumour cells [are] replacing hepatocytes in the liver plates, at the interface or throughout the metastasis, conserving the tissue architecture of the liver, without inflammation or fibrosis. Tumour cells and hepatocytes [have] intimate cell – cell contact."
Terayama 2002	"fibrous capsule", note: thickness measured	n/a	n/a
Yamaguchi 2002	n/a	"Metastatic lesions with expansive growth but a sharp boundary with adjacent tissues were classified as INF type alpha"	"Lesions with invasive growth and no boundary with adjacent tissues..."
Vermeulen 2001	"... metastases were separated from the surrounding liver parenchyma by a rim of desmoplastic stroma in which, in all cases, a dense lymphocytic infiltrate was present. Numerous bile ducts and capillaries were also present in the rim of newly formed stroma and often tumour cell nests were seen infiltrating the stroma. The reticulin pattern of the liver parenchyma was not conserved within the metastasis. There was no contact between tumour cells and hepatocytes."	"The liver plates [are] pushed aside and ran in parallel with the circumference of the metastases. There [is] no desmoplastic stroma formation and the tumour cells [are] separated from the hepatocytes by a thin layer of reticulin fibres. A mild inflammatory infiltrate [is] nearly always present at the interface. The reticulin pattern of the liver parenchyma [is] not conserved within the metastasis."	"Tumour cells [are] replacing hepatocytes in the liver plates, conserving the reticulin network of the liver parenchyma. There [is] no inflammation and no fibrosis. Tumour cells and hepatocytes [have] intimate cell–cell contact.
Weber 2001	"Existence of pseudocapsule was established if metastatic tumor was completely surrounded by fibrous tissue"	"When microtubular cancer nests or microscopic cluster of undifferentiated cancer cells were detected ahead of the invasive front with moderate or severe intensity, the type of tumor progression at the tumor edge was diagnosed as infiltrative [...]. When these nests did not exist or were detected with very mild intensity, the type of tumor progression was diagnosed as expansive..."	"When microtubular cancer nests or microscopic cluster of undif- ferentiated cancer cells were detected ahead of the invasive front with moderate or severe intensity, the type of tumor progression at the tumor edge was diagnosed as infiltrative..."
Lunevicius 2001	The degree of fibrotic tissue layer between the periphery of metastatic nodules and the hepatic parenchyma was graded based on the thickness of fibrotic bundles. A fibrotic bundle at the margin of a metastatic nodule with approximately 0.5 mm or more regular thickness around the entire surface of liver metastasis was designated as 'capsule'.'[...] 'Non-capsule\' was defined as the virtual absence of a fibrous band around the metastasis in which tumor cells faced the hepatic parenchyma directly. A thin fibrous tissue layer was defined as 'intermediate' between capsule and non-capsule"	n/a	n/a
Okano 2000	"...fibrous tissue between tumors and the liver parenchyma was classified as [...] none: no fibrous tissue observed; thin: tumor was separated by several layers of collagen bundles in histologic sections; thick: tumor was separated by 10 or more layers of such bundles [...]. Each tumor was categorized into three types (none, thin, and thick) based on the predominant features of fibrous pseudocapsule. Variations in the thickness of fibrous pseudocapsule in each tumor were observed to varying extents. However, the histologic features of fibrous pseudocapsule were so characteristic that each tumor could be categorized easily according to the predominant histologic features. A pseudocapsule did not always surround the entire periphery. "	n/a	n/a
Ambiru 1999	"The pseudocapsule was histologicallydefined as fibrous tissue between the hepatic tumor and surrounding hepatic parenchyma"	n/a	n/a
Nagashima 1999	"Marginal fibrosis [...] when the margin of the hepatic tumor was dominantly replaced by fibrosis"	..."expansive growth of hepatic tumor [...] when the hepatic tumor was pushing out the normal liver tissue and reasonably well circumscribed"	"the hepatic tumor invaded in a diffuse manner with widespread penetration of normal liver tissues"
Terayama 1996	"enclosing fibrous capsule"	"tumor cells compress the liver-cell plates and sinusoids and make the liver cells atrophic. In this pattern, the border of the tumor is somewhat even and smooth."	"penetration of normal liver tissue"
Yamamoto 1995	"…fibrous tissue between tumors and the liver parenchyma was classified as follows: none-no fibrous tissue observed; thin-tumor was separated by several layers of collagen bundles; thick-tumor was separated by ten or more layers of such bundles"	n/a	n/a
Morino 1991	"capsule formation"	"the cancer cells are seen compressing the hepatocytes along the liver cell cord. There is no destruction of normal hepatocytes."	"sinusoidal": "cancer cells invade sinosoids and destroy the normal hepatocytes"

Supplementary Table 2: Outcome analysis in eligible studies that assessed at least one outcome item

study	outcome assessed, groups compared, findings	outcome snap
Dam 2017	OS, univariate and multivariate analysis; in both, replacement associated with significantly inferior survival (univariate HR [95% CI] for replacement 1.56 [1.186–2.05], multivariate HR 1.73 [1.28–2.33], p=0.019 and p<0.001, respectively). Direct comparison of OS in desmoplastic vs replacement growth patterns, stratified by predominant (>50%) pattern; consistently superior outcome in patients with desmoplastic growth pattern (log-rank p=0.006).	favours desmoplastic disfavors replacement
Frentzas 2016, London cohort	In univariate analysis, only the growth patterns showed associations with pathological response (p<0.001, X2 test) with a higher number of lesions with <25% viable tumor cells in the replacement compared to the encapsulated pattern	favors desmoplastic disfavors replacement
Frentzas 2016, Montréal cohort	Better overall survival after Bev-Chemo in patients with encapsulated growth pattern, sampled after neoadjuvant chemo, HR = 3.50, 95% CI 1.49–8.20 (Cox regression) p = 0.0022 (log–rank); n=61 patients; no differences without Bev, HR = 0.90, 95% CI 0.31–2.61 (Cox regression) p = 0.846 (log–rank); n=28 patients. In uni- and multivariate analysis of patients treated with Bev-Chemo, the growth patterns were the only significant finding, (p = 0.004 in uivariate, p=0.0135 in multivariate analysis, X2), favouring the encapsulated type	favors desmoplastic
Siriwardana 2016	5y-OS: 84% in encapsulated CRLM, and 46% in infiltrative CRLM (p=0.044, Cox regression), n=25 patients. An additional survival rate is reported, presumably over a shorter period of follow-up, which showed similar values, albeit failing to reach the level of significance.	favors desmoplastic disfavors replacement
Serrablo 2016	5y actuarial survival and 5y DFS: No differences between tumor with and without "pseudocapsule", or those with a replacement or pushing pattern.	no differences
Eefsen 2015b	RFS, univariate & multivariate analysis: replacement and mixed significant (HR 1.93/2.16, p=0.010/0.003 and 1.78/1.70, p=0.006/0.013 respectively). OS, multivariate: replacement and mixed significant (HR 2.26, p=0.016; HR1.72;p=0.047, respectively)	favors desmoplastic disfavors replacement
Brunner 2014	Kaplan – Meier analysis: encapsulated CRLM associated with longer survival than non-encapsulated (median 64 vs 31 months; HR 2.53, p < 0,001), also in R0 resected patients.	favors desmoplastic
Pinheiro 2014	Overall recurrence, univariate logistic regression: replacement growth pattern not significantly prognostic (OR 2.13, p=0.11). Overall recurrence and intrahepatic recurrence, multivariate logistic regression: replacement patter at level of significance, OR 2.81. p=0.05 for overall recurrence and significantly associated with intrahepatic recurrence (OR 3.50, p=0.02). 5y-DFS: replacement inferior to pushing, p = 0.05	disfavors replacement
Wiggans 2012	1y recurrence: presence of pseudocapsule associated with lower frequency of recurrence (Incidence ratio 0.3, p = 0.030)	favors desmoplastic
Nyström 2012	OS of pushing 63.9 +/-6.9 months, vs for desmoplastic 93 +/- 10.5 months, p=0.1, significant when considering only pts with follow-up of >60months	favors desmoplastic (over pushing)
Van Den Eynden 2012	median survival in months (95% CI): replacement 43 (30.1 - 55.9) pushing 20.5 (17.6 - 23.4) desmoplastic 38.4 (25.2-51.6) mixed 28.2 (14.7-41.6) no significant differences in OS over whole study period, pushing growth pattern worse when considering first 2 years Note: In revised classification, it can be assumed that most "pushing"-type CRLM would be grouped into the "replacement" type, Frentzas 2016	disfavors pushing
Rajaganeshan 2007a	non-significant for recurrence following R1 resection between "encapsulated" and "non-encapsulated". OS: Encapsulated/non: 0.33 DFS: Encapsulated/non: 0.91	no differences
Yamaguchi 2002	DFS: "IFNy" (resembling replacement type) significantly worse than other patterns (p < 0.01)	disfavors replacement
Weber 2001	univariate analysis, median OS: 2.9ys for "pseudocapsule absent", and 4.2ys for "pseudocapsule present", p=0.0456. OS 3.9ys for "expansive" vs 2.8ys for "infiltrative", logrank p 0.034. multivariate analysis of DFS yielded ki67 labelling index and microscopic GP (p=0.034) as significant	favors desmoplastic disfavors replacement
Lunevicius 2001	"survival rate" overall not different in encapsulated vs non-encapsulated CRLM (log-rank p=0.163). Recurrence in the liver significantly less frequent in encapsulated, p <0.05.	favors desmoplastic
Okano 2000	"proportion surviving", univariate analysis: significantly better in CRLM with pseudocapsule, best with thick pseudocapsule (p<0.001).	favors desmoplastic
Ambiru 1999	OS, univariate analysis: survial at 5ys 23 months in non-encapsulated CRLM vs 42 months in encapsulated CRLM, o = 0.0244). Remained significant in multivariate analysis.	favors desmoplastic
Nagashima 1999	univariate analzsis, "cancer-related survival"; mean survival 39.6 months for pushing CRLM, 12.2 months for replacement CRLM, p=0.0001 In encapsulated 33.2 months vs 10.6 months, p=0.0072	favors desmoplastic disfavors replacement
Morino 1991	"cumulative survival rate" up to 4.5y follow up: significantly better survival in encapsulated vs non-encapsulated CRLM	favors desmoplastic